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(54) Title: ANTIVIRAL PHOSPHONO-ALKEN DERIVATIVES OF PURINES

(57) Abstract

Purine derivatives, a process for their preparation and their use as antiviral agents.

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ANTIVIRAL PHOSPHONO-ALKEN DERIVATIVES OF PURINES

The present invention relates to compounds having antiviral activity, to processes for their preparation and to their use as pharmaceuticals.

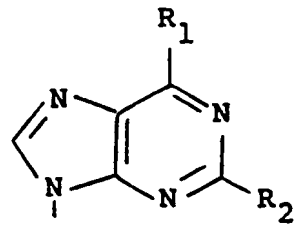
Coll. Czech. Chem. Commun., 1988, 53, 2753 (Rosenberg et. al.) describes phosphonylalkyl derivatives of adenine.

EP-A-343133 (Medivir Aktiebolag) discloses a group of phosphonylalkyl purine derivatives which are described as having antiviral activity.

EP-A-404296 (Beecham group p.l.c.), published 27.12.90, describes a group of phosphonylalkoxy purine derivatives having antiviral activity.

A novel, structurally distinct class of compounds has now been discovered, these compounds being phosphonylalkenyl or phosphonylalkenyloxy derivatives of purine, and also having antiviral activity.

Accordingly, the present invention provides a compound of formula (I), or a pharmaceutically acceptable salt thereof:



$R_4HC{=}CHCHR_3X$

(I)

wherein

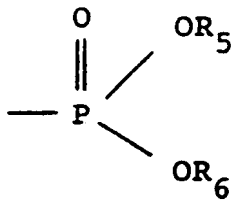
X is $-CH_2O$ or $-CH_2$;

R_1 is hydroxy or amino;

R_2 is hydrogen or amino;

R_3 is hydrogen, hydroxymethyl or acyloxymethyl; and

R_4 is a group of formula:



wherein

R_5 and R_6 are independently selected from hydrogen, C_{1-6} alkyl and optionally substituted phenyl.

When R_1 is hydroxy and R_2 is amino, the compound of formula (I) is a guanine derivative;

When R_1 is amino and R_2 is hydrogen, the compound of formula (I) is an adenine derivative;

When R_1 is hydroxy and R_2 is hydrogen, the compound of formula (I) is a hypoxanthine derivative; and

When R_1 and R_2 are both amino groups, the compound of formula (I) is a 2,6-diaminopurine derivative.

Often, the compound of formula (I) is a guanine or adenine derivative.

Suitable examples of the acyl group in R_3 when acyloxymethyl, include carboxylic acyl, such as C_{1-7} alkanoyl and benzoyl optionally substituted in the phenyl ring as defined below for R_5/R_6. Preferred acyl groups include acetyl, propionyl, butyryl, heptanoyl and hexanoyl.

Suitable examples of R_5 and R_6 include hydrogen, methyl, ethyl, n- and iso-propyl, n-, sec-, iso- and tert-butyl, and phenyl optionally substituted by one, two or three groups or atoms selected from halogen, such as fluoro, chloro, bromo, and C_{1-4} alkyl or C_{1-4} alkoxy wherein the alkyl moiety is selected from those listed for R_5/R_6 above.

Examples of pharmaceutically acceptable salts of the compound of formula (I) are acid addition salts formed with a pharmaceutically acceptable acid such as hydrochloric acid, orthophosphoric acid and sulphuric acid. Pharmaceutically acceptable salts also include those formed with organic bases, preferably with amines, such as ethanolamines or diamines; and alkali metals, such as sodium and potassium.

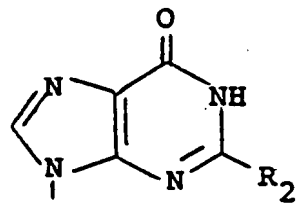
As the compound of formula (I) contains a phosphonate group, suitable salts include metal salts, such as alkali metal salts, for example sodium or potassium, alkaline earth metal salts such as calcium or magnesium and ammonium or substituted ammonium salts, for example those with lower alkylamines such as triethylamine, hydroxy-lower alkylamines such as 2-hydroxyethylamine, bis-(2-hydroxyethyl)amine or tris-(2-hydroxyethyl)amine.

It will be appreciated that some of the compounds of formula (I), especially those wherein R_3 is other than hydrogen, have an asymmetric centre, and therefore are capable of existing in more than one stereoisomeric form. The invention extends to each of these forms individually and to mixtures thereof, including racemates. The isomers may be separated conventionally by chromatographic methods or using a resolving agent. Alternatively, the individual isomers may be prepared by asymmetric synthesis using chiral intermediates.

It will also be appreciated that, since the compounds of formula (I) contain a $R_4HC=CH$ moiety, they are capable of existing in E and Z (trans and cis) forms. The invention extends to each of these forms and to mixtures thereof.

The compounds of formula (I) including their alkali metal salts may form solvates such as hydrates and these are included wherever a compound of formula (I) or a salt thereof is herein referred to.

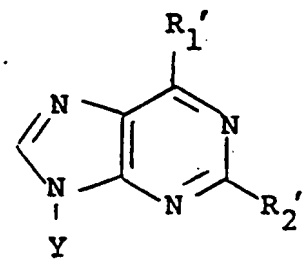
It will be appreciated that, when R_1 is hydroxy in formula (I) the compound exists in the predominant tautomeric form of structure (IA):



$R_4HC=CHCHR_3X$

(IA)

The invention also provides a process for the preparation of a compound of formula (I), or a pharmaceutically acceptable salt thereof, which process comprises condensing a compound of formula (II):



(II)

with a side chain intermediate of formula (III):

$$R_4'HC=CHCHR_3'CH_2OH \qquad (III)$$

wherein, when X is $-CH_2O$ in formula (I), Y is OH and, when X is $-CH_2$, Y is H; R_1', R_2', R_3' and R_4' are R_1, R_2, R_3 and R_4 respectively, or groups or atoms convertible thereto; and thereafter, when desired or necessary, converting R_1', R_2', R_3' and/or R_4', when other than R_1, R_2, R_3 and/or R_4 to R_1, R_2, R_3 and/or R_4 respectively, and/or converting R_1', R_2', R_3' and/or R_4' when R_1, R_2, R_3 and/or R_4, to other R_1, R_2, R_3 and/or R_4, and/or forming a pharmaceutically acceptable salt thereof.

The reaction takes place in the presence of a dehydrating catalyst, such as diethyl azodicarboxylate in the presence of triphenylphosphine.

Examples of conversions of variable groups are as follows:

$\underline{R_1'-R_1}$

a) An R_1 hydroxy group may be converted to R_1' is chloro, by chlorination using a reagent such as phosphorus oxychloride, preferably in the presence of tetraethylammonium chloride and dimethylaniline (as acid acceptor) in CH_3CN at reflux temperatures, according to the method described by M.J. Robins and B. Ozanski, Can. J. Chem, 59, 2601 (1981).

b) An R_1' chloro group may be converted to R_1 is hydroxy by hydrolysis using aqueous mineral acid, such as hydrochloric acid, or more preferably, using an organic acid, such as formic acid at elevated temperature, suitably 70-150°C, preferably around 100°C.

c) An R_1' chloro group may be converted to R_1 is amino by treatment with ammonia in a lower alkanol, such as ethanol or methanol in an autoclave at 100°C for a period of about 7 hours, or alternatively, by treatment with sodium azide in dimethylformamide (forming an R_1 is N_3 intermediate), followed by reduction with ammonium formate/palladium on charcoal, in methanol or with triphenylphosphine in water as described by Vaulter et al., Tet. Letts. 24(8) 763-764(1983).

d) An R_1' alkoxy group, such as methoxy, may be converted to R_1 hydroxy by the methods of D.R. Haines, J. Med. Chem. 1987, 30, 943 and K.K. Ogilvie and H.R. Hanna, Can. J. Chem. 1984, 62, 2702, or using trimethylsilyl bromide, as described in Example 1b) hereinafter.

e) An R_1' protected amino group, such as tritylamino, may be converted to amino, by treatment with aqueous acetic acid, preferably 80% acetic acid at elevated temperature, around 80°C. R_1' may also be phthalimido, which may be converted to amino by treatment with methyl hydrazine or hydrazine in an inert solvent, such as dichloromethane, at ambient temperature.

R_2'-R_2

a) R_2' may be protected amino, such as formylamino, which may be converted to R_2 is amino by hydrolysis; or R_2' may be di-t-butyloxycarbonylamino.

R_3'-R_3

a) Hydroxymethyl may be converted to acyloxy or acyloxymethyl respectively by conventional acylation procedures.

b) Protected hydroxymethyl may be converted to hydroxymethyl by conventional deprotection methods.

Suitable examples of protecting groups and their removal, are as described in EP-A-242482. A particularly suitable protecting group is the t-butyldiphenylsilyl group removable by conventional methods.

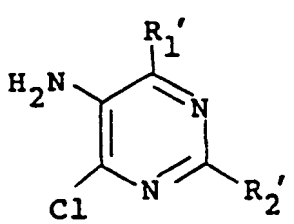
R_4'-R_4

When R_5 and R_6 in R_4 are other than hydrogen, they may be converted to R_5 and R_6 are hydrogen, using a deesterifying reagent, such as trimethylsilyl bromide in an aprotic solvent such as dichloromethane or dimethylformamide at ambient temperature, as described by C.E. McKenna et. al., J.C.S., Chem. Comm., 1979, 739.

Selective conversion of one of R_5 and R_6 to hydrogen, may be achieved by treatment with hydroxide ion, as described by Rabinowitz JACS, 1960, 82, 4564.

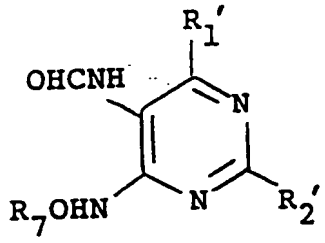
It will be appreciated that the above conversions may take place in any desired or necessary order, having regard to the final desired compound of formula (I).

Compounds of the formula (II) wherein Y is OH are prepared as described in EP-A-313289 and EP-A-319228 (both Beecham Group p.l.c.), from compounds of formula (IV) wherein the 5-amino group is formylated:



(IV)

by reaction with R_7ONH_2 wherein R_7 is a protecting group, to give a compound of formula (V):

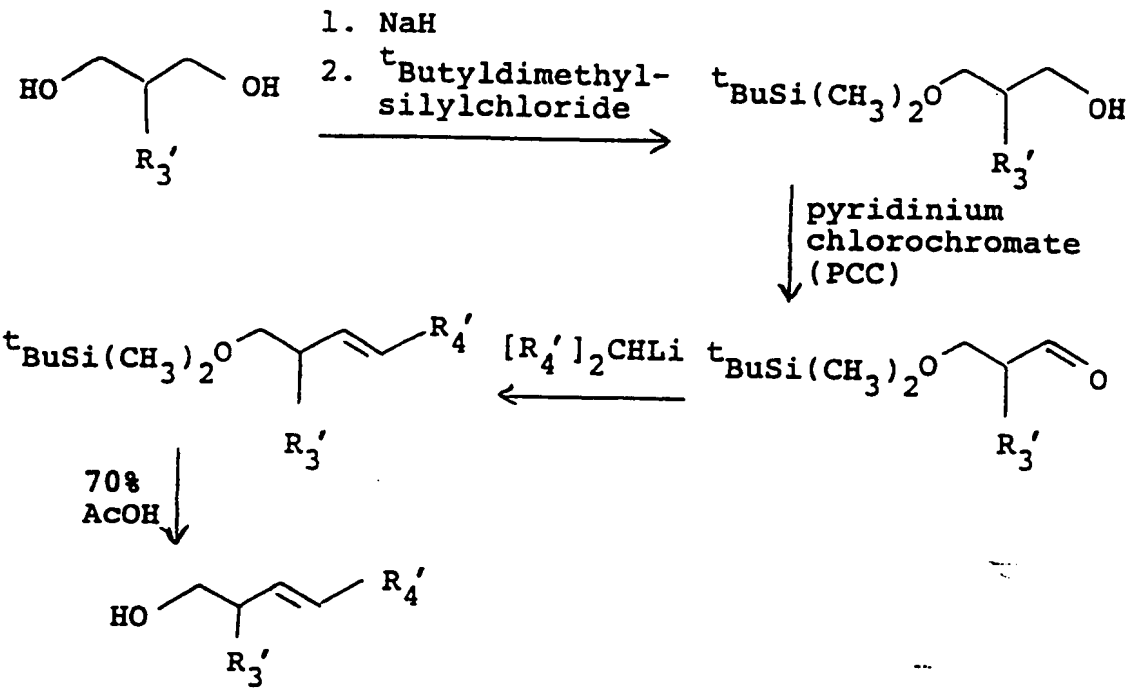


(V)

which may be cyclised with diethoxymethyl acetate, to give a compound of formula (II) wherein the OH group is protected. Suitable values for R_7 include benzyl, removable by hydrogenation, and the tetrahydropyran-2-yl group removable by treatment with 80% acetic acid, at ambient temperature.

Compounds of the formula (II) wherein Y is H are generally known, for example, 2-amino-6-chloropurine may be prepared as described in EP-A-203685 (Beecham Group p.l.c.).

Intermediates of the formula (III) (E-isomers) may be prepared as follows:-



R_4' in the above is a value of R_4, usually wherein R_5 and R_6 are other than hydrogen. R_3' is hydrogen or protected hydroxymethyl.

Intermediated of the formula (III) (Z-isomers) may be prepared as described in Description 2 hereinafter.

When R_3 is hydroxymethyl, appropriate selective protection on one of the hydroxy groups in the side chain intermediate of formula (III) is required, eg using acetate; or the ^{t}butyl dimethylsilyl protecting group may be replaced by the isopropylidine joined together with R_3'.

Intermediates of the formula (III) wherein R_4' is R_4 as defined in formula (I), are novel and form an aspect of the

invention.

Pharmaceutically acceptable salts may be prepared in conventional manner, for example, in the case of acid addition salts, by reaction with the appropriate organic or inorganic acid.

It will be appreciated that the invention provides a process for the preparation of a compound of formula (I) wherein R_3 is hydroxymethyl which process comprises the deprotection of a corresponding compound of formula (I) wherein R_3 is protected hydroxymethyl.

Preferred methods for deprotection, as hereinbefore described, include removal of the acetyl group.

The invention also provides a process for the preparation of a compound of formula (I) wherein R_5 and R_6 are both hydrogen, which process comprises the deesterification of a corresponding compound of formula (I) wherein R_5 and R_6 are the same alkyl or optionally substituted phenyl group.

It will be appreciated that, in some circumstances, it may be possible to prepare the compounds of formula (I) by methods analogous to those generally described in EP-A-404296 (Beecham Group p.l.c.) having regard to the unsaturated side chain and the need for protection of the unsaturated moiety and/or modification of reaction conditions.

The compounds of the invention are of potential use in the treatment of infections caused by viruses, in particular DNA viruses and retroviruses. Examples of DNA viruses include herpesviruses such as herpes simplex types 1 and 2, varicella-zoster virus, Epstein-Barr virus and cytomegalovirus. Examples of retroviruses include lentiviruses such as visna virus, feline immunodeficiency

virus and human immunodeficiency virus (strains 1 and 2).

The compounds may also be inhibitors of tumorogenic viruses and/or of potential use in the treatment of neoplastic diseases, i.e. cancer.

Compounds of the invention may be formulated for use in a pharmaceutical composition. Accordingly, in a further aspect of the invention, there is provided a pharmaceutical composition which comprises a compound of formula (I) or pharmaceutically acceptable salt thereof together with a pharmaceutically acceptable carrier or excipient.

A composition which may be administered by the oral route to humans may be compounded in the form of a syrup, tablet or capsule. When the composition is in the form of a tablet, any pharmaceutical carrier suitable for formulating such solid compositions may be used, for example magnesium stearate, starch, lactose, glucose, rice, flour and chalk. The composition may also be in the form of an ingestible capsule, for example of gelatin, to contain the compound, or in the form of a syrup, a solution or a suspension. Suitable liquid pharmaceutical carriers include ethyl alcohol, glycerine, saline and water to which flavouring or colouring agents may be added to form syrups. The compounds may also be presented with a sterile liquid carrier for injection.

The composition may also be formulated for topical application to the skin or eyes.

For topical application to the skin, the composition may be in the form of a cream, lotion or ointment. These formulations may be conventional formulations well known in the art, for example, as described in standard books of

pharmaceutics and cosmetics, such as Harry's Cosmeticology published by Leonard Hill Books and the British Pharmacopaeia.

The composition for application to the eyes may be a conventional eye-drop composition well known in the art, or an ointment composition.

Preferably, the composition of this invention is in unit dosage form or in some other form that may be administered in a single dose. A suitable dosage unit might contain from 50 mg to 1 g of active ingredient, for example 100 to 500 mg.

Such doses may be administered 1 to 4 times a day or more usually 2 or 3 times a day. The effective dose of compound will in general be in the range of from 1.0 to 20 mg/kg of body weight per day or more usually 2.0 to 10 mg/kg per day.

No unacceptable toxicological effects are indicated at the above described dosage levels.

The invention also provides a method of treating viral infections in a human or non-human animal, which comprises administering to the animal an effective, non-toxic amount of a compound of formula (I) or a pharmaceutically acceptable salt thereof.

The invention also provides a compound of formula (I) or a pharmaceutically acceptable salt thereof for use as an active therapeutic substance, in particular for the treatment of viral infections.

The compounds of the invention are also believed to exhibit a synergistic antiherpesvirus effect in conjunction with interferons; and combination products comprising these two

components for sequential or concomitant administration, by the same or different routes, are therefore within the ambit of the present invention.

The following examples illustrate the invention; the following descriptions illustrate the preparation of intermediates.

Description 1 (Intermediates for Examples 1 to 8)

a) 3-(t-Butyldimethylsilyloxy)propan-1-ol

To a suspension of sodium hydride (6.29g, 262mmol) in dry tetrahydrofuran (400ml) was added 1,3-propanediol (20.0g, 262mmol) over 5min and the mixture was stirred at room temperature under dry nitrogen for 1.5hr. t-Butyldimethylsilyl chloride (39.5g, 262mmol) was added portionwise and the mixture was stirred at room temperature for 1.5hr. Saturated sodium chloride solution (300ml) then ether (500ml) were added. The organic portion was dried (magnesium sulphate), filtered and the solvent removed. The residue was purified by column chromatography on silica gel eluting with ether-hexane (1:2, 3:2) to afford 3-(t-butyldimethylsilyloxy)propan-1-ol as a colourless liquid (41.2g, 83%); $\delta_H(CDCl_3)$ 0.10 (6H, s, CH_3), 0.93 (9H, s, $C(CH_3)_3$), 1.80 (2H, qu, J 6 Hz, CH_2), 2.37 (1H, br.s, OH), 3.87 (4H, m, CH_2O).

b) 3-(t-Butyldimethylsilyloxy)propanal

To a suspension of pyridinium chlorochromate (8.50g, 39.4mmol) in dichloromethane (53ml), stirred at room temperature under dry nitrogen, was added 3-(t-butyldimethylsilyloxy)propan-1-ol (5.00g, 26.3mmol). After 1.5hr, dry ether (50ml) was added and the supernatant liquid decanted from a black gum. The residual gum was washed with ether (3 x 50ml) and the combined organic portions passed through a column of Florisil. The resulting brown solution was evaporated then the residue taken up in dichloromethane and passed through fresh Florisil to give a yellow solution from which the solvent was removed to leave a brown liquid (2.75g). This material was shown by ^{1}Hnmr analysis to be approximately 40% pure and was used without further purification; $\delta_H(CDCl_3)$ 0.10 (6H, s, CH_3), 0.93 (9H,

s, $C(CH_3)_3$), 0.63 (2H, dt, J 2 Hz and 6 Hz respectively, CH_2), 4.03 (2H, t, J 6 Hz, CH_2O), 9.97 (1H, d, J 2 Hz, CHO).

c) Diisopropyl (E)-4-(t-butyldimethylsilyloxy)but-1-enylphosphonate

To a solution of tetraisopropyl methylenebisphosphonate (2.50g, 7.26mmol) in n-heptane (50ml) was added n-butyllithium (2.70ml of 2.7M solution in n-hexanes; 7.29mmol) and the mixture stirred at room temperature under dry nitrogen for 15min. To the solution was added crude 3-(t-butyldimethylsilyloxy)propanal (approx. 5.85mmol) and the mixture heated under reflux for 0.5hr then stirred at room temperature for 64hr. The mixture was filtered then the solvent removed. The residue was purified by column chromatography on silica gel eluting with dichloromethane-ethyl acetate (9:1, 4:1) to afford diisopropyl (E)-4-(t-butyldimethylsilyloxy)but-1-enylphosphonate as a colourless oil (1.10g, 43%); ν_{max} (film) 2940, 1625, 1460, 1380, 1250, 1105, 980 and 830cm^{-1}; $\delta_H(CDCl_3)$ 0.03 (6H, s, $SiCH_3$), 0.90 (9H, s, $C(CH_3)_3$), 1.32 (12H, dd, J 3 Hz and 6 Hz, $CH(C\underline{H}_3)_2$), 2.43 (2H, m, CH_2), 3.72 (2H, t, J 7 Hz, CH_2O), 4.65 (2H, m, $C\underline{H}(CH_3)_2$), 5.72 (1H, dd, J 17 Hz and 20 Hz, $PC\underline{H}$=CH), 6.75 (1H, ddt, J 7 Hz, 17 Hz and 20 Hz, PCH=C$\underline{H}$); FABMS(thioglycerol) 351 (MH^+) (Found: C, 54.76; H, 10.05%. $C_{16}H_{35}O_4PSi$ requires C, 54.84; H, 10.07%).

d) Diisopropyl (E)-4-hydroxybut-1-enylphosphonate

A solution of diisopropyl(E)-4-(t-butyldimethylsilyloxy)but-1-enylphosphonate (0.84g, 2.40mmol) in acetic acid-water (2:1)(10ml) was stirred at 70°C for 2hr. The solvent was removed and the residue purified by column chromatography on silica gel eluting with acetone-hexane (1:1) to give diisopropyl (E)-4-hydroxybut-1-enylphosphonate

as a gum (0.43g, 76%); ν_{max} (film) 3380, 2970, 1625, 1460, 1380, 1370, 1220 and 980cm^{-1}; δ_H($CDCl_3$) 1.32 (12H, dd, J 9 Hz and 6 Hz, $CH(C\underline{H}_3)_2$), 1.85 (1H, br.s, OH), 2.50 (2H, m, CH_2), 3.76 (2H, t, J 6 Hz, CH_2O), 4.69 (2H, m, $C\underline{H}(CH_3)_2$), 5.80 (1H, dd, J 16 Hz and 18 Hz, PC<u>H</u>=CH), 6.75 (1H, ddt, J 7 Hz, 17 Hz and 22 Hz, PCH=C<u>H</u>); CIMS (isobutane) 237 (MH^+).

<u>Description 2</u> (Intermediate for Examples 9, 10 and 11)

a) <u>Diethyl (Z)-4-(t-butyldimethylsilyloxy)but-1-enylphosphonate</u>

To a solution of <u>n</u>-butyllithium in hexane (38.9ml, 2.7M, 105mmol) stirred at -20°C under dry nitrogen was added a solution of diisopropylamine (11.5g, 114mmol) in dry THF (70ml). The solution was cooled to -70°C before a solution of diethyl methylphosphonate (7.6g, 50mmol) in dry THF (10ml) was added dropwise. A solution of chlorotrimethylsilane (5.8g, 53mmol) in dry THF (15ml) was then added dropwise, maintaining the internal temperature below -60°C. The resulting solution was stirred at -70°C for 15min. then warmed to -20°C before a solution of 3-(<u>t</u>-butyldimethylsilyloxy)propanal (approx. 46mmol) in dry THF (10ml) was added dropwise. The solution was then stirred at room temperature for 1.5hr. The reaction mixture was neutralized by addition of 2M hydrochloric acid and extracted with ether (250ml). The organic phase was dried (magnesium sulphate), filtered and the solvent removed. The residual oil was purified by column chromatography on silica gel eluting with hexane-acetone (5:1, 3:1) to afford diethyl (<u>Z</u>)-4-(<u>t</u>-butyldimethylsilyloxy)but-1-enylphosphonate as a colourless liquid (1.5g, 9%); ν_{max} (film) 2940, 1625, 1390, 1245, 1095, 1055, 1030 and 950cm^{-1}; δ_H($CDCl_3$) 0.05 (6H, s, $SiCH_3$), 0.87 (9H, s, $C(CH_3)_3$), 1.30 (6H, t, J 7Hz, CH_3), 2.83 (2H, m, CH_2), 3.73 (2H, t, J 7Hz, CH_2OSi), 4.10 (4H, qu, J 7Hz, CH_2O), 5.70 (1H, dd, J 14Hz and 20Hz, PC<u>H</u>=CH),

6.70 (1H, ddt, J 7Hz, 14Hz and 54Hz, PCH=CH) (Found: MH^+ 323.1808. $C_{14}H_{31}O_4PSi$ requires MH^+ 323.1808).

b) Diethyl (Z)-4-hydroxybut-1-enylphosphonate

A solution of diethyl (Z)-4-(t-butyldimethylsilyloxy)but-1-enylphosphonate (1.32g, 4.09mmol) in acetic acid-water (2:1) (35ml) was stirred at room temperature for 2hr. The solvent was removed and the residue was purified by column chromatography on silica gel eluting with dichloromethane-methanol (19:1) to give diethyl (Z)-4-hydroxybut-1-enylphosphonate as a colourless liquid (0.5g, 59%); ν_{max} (film) 3380, 2980, 1720, 1620, 1390, 1230 and 1020cm^{-1}; $\delta_H(CDCl_3)$ 1.33 (6H, t, J 7Hz, CH_3), 2.70 (2H, m, CH_2), 3.15 (1H, s, OH), 3.73 (2H, t, J 7Hz, CH_2O), 4.00 (4H, qu, J 7Hz, CH_3CH_2), 5.70 (1H, dd, J 14Hz and 20Hz, PCH=CH), 6.60 (1H, ddt, J 7Hz, 14Hz and 54Hz, PCH=CH) (Found: MH^+ 209.0942. $C_8H_{17}O_4P$ requires MH^+ 209.0943).

Description 3 (Intermediates for Examples 12-18)

a) Diisopropyl (E)-2-(1,3-dioxan-5-yl)ethenylphosphonate

A solution of 2-(1,3-dioxan-5-yl)ethanol (2g, 14mmol) in dichloromethane (5ml) was added dropwise to pyridinium chlorochromate (4.4g, 20mmol) in dichloromethane (30ml). The mixture was stirred at room temperature for 2h, then treated with ether (30ml). After stirring for a further 10min, at room temperature, the mixture was filtered through silica, the residue extracted with ether (50ml), filtered and the combined filtrates evaporated in vacuo to give an oil (0.75g) which was shown by 90MHz n.m.r. to contain 60% aldehyde (23%).

A solution of tetraisopropyl methylenebisphosphonate (1g, 3.1mmol) in heptane (25ml) was treated with 2.7M

n-butyllithium in hexane (1.1ml, 3.1mmol). After stirring at room tempeature for 15min, the aldehyde obtained above (0.75g, 60% pure, 3.1mmol), suspended in heptane (5ml) was added. After stirring at room temperature for 15min, the solvent was removed and the residue chromatographed on silica gel, eluting with acetone-hexane (1:4) to give diisopropyl (E)-2-(1,3-dioxan-5-yl)ethenylphosphonate as an oil (0.88g, 98%); υ_{max} (KBr) 3386, 2979, 2938, 2870, 1740, 1627, 1470, and 1455cm^{-1}; δ_H($CDCl_3$) 1.30 (6H, d, J 6Hz, 2xCH_3CH), 1.33 (6H, d, J 6Hz, 2xCH_3CH), 1.42 (3H, s, CH_3), 1.44 (3H, s, CH_3), 2.65 (1H, m, CH), 3.85 (4H, m, 2xCH_2), 4.55 [2H, m, 2xCH(CH_3)$_2$], 5.79 (1H, ddd, J 1, 17 and 19Hz, PCH=CH), 6.60 (1H, ddd, J 7, 17 and 22Hz, PCH=CH) (Found: C, 55.03; H, 8.94%. $C_{14}H_{27}O_5P$ requires C, 54.89; H, 8.88%).

b) Diisopropyl (E)-4-hydroxy-3-hydroxymethylbut-1-enylphosphonate

A solution of diisopropyl (E)-2-(1,3-dioxan-5- yl)-ethenylphosphonate (0.73g, 2.4mmol) in 3% methanolic HCl (10ml) was stirred at room temperature for 1.5h. The solvent was removed in vacuo and the residue chromatographed on silica gel eluting with ethyl acetate, increasing polarity to ethyl acetate-methanol (20:1) to give diisopropyl (E)-4-hydroxy-3-hydroxymethylbut-1-enylphosphonate as an oil (0.4g, 63%): υ_{max} (film) 3391, 2979, 2933, 2877, 1738, 1630, 1467, and 1454cm^{-1}; δ_H ($CDCl_3$) 1.32 (6H, d, J 6Hz, 2xCH_3CH), 1.32 (6H, d, J 6Hz, 2xCH_3CH), 2.61 (1H, m, CH), 3.40 (2H, br.s, D_2O exchangeable OH's), 3.80 (4H, m, 2xCH_2OH), 4.65 [2H, m, 2xCH(CH_3)$_2$], 5.82 (1H, ddd, J 1, 17 and 20Hz, PCH=CH), 6.71 (1H, ddd, J 7, 17 and 23Hz, PCH=CH) (Found: C, 49.52; H, 9.04%. $C_{11}H_{23}O_5P$ requires C, 49.62; H, 8.71%).

c) Diisopropyl (E)-3-acetoxymethyl-4-hydroxybut-1-enylphosphonate

A solution of diisopropyl (E)-4-hydroxy-3-hydroxymethylbut-1-enylphosphonate (5g, 19mmol), trimethyl orthoacetate (7ml, 56mmol) and p-toluenesulphonic acid (0.36g, 1.9mmol) in anhydrous THF (50ml) was stirred at room temperature for 1.5h. The solution was treated with water (5ml), stirred for a further 30 min, then treated with triethylamine (0.1ml). The solvent was removed in vacuo and the residue chromatographed on silica, eluting with chloroform-methanol (30:1) to give diisopropyl (E)-3-acetoxymethyl-4-hydroxybut-1-enylphosphonate as an oil (4.94g, 85%); υ_{max} (film) 3382, 2980, 2934, 2877, 2361, 2333, 1741, 1631, 1468, and 1455 cm^{-1}; $\delta_H(CDCl_3)$ 1.30 (6H, d, J 6.3Hz, 2x$C\underline{H}_3CH$), 1.34 (6H, d, J 6Hz, 2x$C\underline{H}_3CH$) 2.06 (3H, s, CH_3CO), 2.40 (1H, br.s, D_2O exchangeable OH), 3.68 (2H, m, $C\underline{H}_2OH$), 4.24 (2H, m, CH_2), 4.64 [2H, m, 2x$C\underline{H}(CH_3)_2$], 5.83 (1H, ddd, J 1, 17 and 19Hz, P$C\underline{H}$=CH), 6.67 (1H, ddd, J 8, 17 and 22Hz, PCH=C$\underline{H}$) (Found: C, 50.15; H, 8.46%; MH^+ 309.1466. $C_{13}H_{17}O_6P.0.25\ H_2O$ requires: C, 49.95; H, 8.22%; MH^+ 309.1467).

d) Diisopropyl (E)-3-acetoxymethyl-4-t-butyl-diphenylsilyloxybut-1-enylphosphonate

To a solution of diisopropyl (E)-3-acetoxymethyl-4-hydroxybut-1-enylphosphonate (3g, 9.7mmol) and imidazole (1.7g, 25mmol) in anhydrous THF (60ml) at 0°C was added t-butyldiphenylsilylchloride (3.2ml, 12.7mmol). After stirring at room temperature for 3h, the solvent was removed and the residue was partitioned between chloroform (100ml) and brine (30ml). The organic phase was dried ($MgSO_4$), evaporated in vacuo and chromatographed on silica

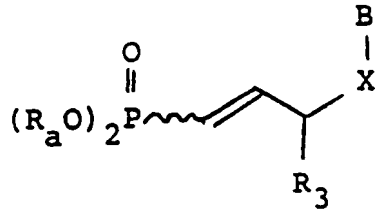
gel, eluting with chloroform of increasing polarity to chloroform-methanol (100:1) to give diisopropyl (E)-3-acetoxymethyl-4-t-butyldiphenylsilyloxybut-1-enyl-phosphonate as an oil (5g, 94%); υ_{max} (film) 3071, 3050, 2977, 2931, 2858, 1743, 1630, 1582, 1472, and 1425cm^{-1}; δ_H ($CDCl_3$) 1.05 [9H, s, $C(CH_3)_3$], 1.26 (3H, d, J 6Hz, CH_3CH), 1.27 (3H, d, J 6Hz, CH_3CH), 1.32 [6H, d, J 6.3Hz, $(CH_3)_2CH$], 1.98 (3H, s, CH_3CO), 2.74 (1H, m, CH), 3.72 (2H, m, CH_2), 4.22 (2H, m, CH_2), 4.65 (2H, m, $2xCH(CH_3)_2$], 5.76 (1H, ddd, J 1, 17 and 18Hz, PCH=CH), 6.99 (1H, ddd, J 7, 17 and 22Hz, PCH=CH), 7.3-7.7 (10H, m, $2xC_6H_5$) (Found: C, 63.42; H, 8.22%. $C_{29}H_{43}O_6PSi$ requires C, 63.71; H, 7.93%.).

e) Diisopropyl (E)-3-(t-butyldiphenylsilyloxy)methyl-4-hydroxybut-1-enylphosphonate

A solution of diisopropyl (E)-3-acetoxymethyl-4-t-butyldiphenylsilyloxybut-1-enylphosphonate (5g, 9.2mmol) in methanol (50ml) was stirred with potassium carbonate (63g, 0.45mmol) for 5h at room temperature. The solvent was removed in vacuo and the residue was chromatographed on silica gel eluting with chloroform-methanol (100:1) of increasing polarity to (30:1) to give diisopropyl (E)-3-t-butyldiphenylsilyloxymethyl-4-hydroxybut-1-enylphosphonate as an oil (3.4g, 73%): υ_{max} (film) 3381, 3071, 3025, 2940, 2931, 2858, 2360, 2332, 1631, 1585, 1471 and 1428cm^{-1}; δ_H ($CDCl_3$) 1.05 [9H, s, $C(CH_3)_3$], 1.25 (3H, d, J 6Hz, $CHCH_3$), 1.27 (3H, d, J 6.1Hz, $CHCH_3$), 1.31 [6H, d, J 6Hz, $CH(CH_3)_2$], 2.15 (1H, t, J 5.9Hz, D_2O exchangeable OH), 2.65 (1H, m, CH), 3.80 (4H, m, $2xCH_2$), 4.65 [2H, m, $2xCH(CH_3)_2$], 5.76 (1H, ddd, J 1, 17 and 19Hz, PCH=CH), 6.64 (1H, ddd, J 8, 17 and 23Hz, PCH=CH), 7.4-7.7 (10H, m, $2xC_6H_5$) (Found: C, 63.65; H, 8.16%. M^+ 504.2444. $C_{27}H_{41}O_5PSi.0.25\ H_2O$ requires C, 63.69; H, 8.22%. M^+ 504.2461).

Examples

The following compounds were prepared:



A = adenine
G = guanine
D = 2,6-diaminopurine

Example No.	B	R_a	R_3	X	Isomer
1	G	H	H	CH_2O	E
2	A	^iPr	H	CH_2O	E
3	A	H	H	CH_2O	E
4	A	^iPr	H	CH_2	E
5	A	H	H	CH_2	E
6	G	H	H	CH_2	E
7	D	^iPr	H	CH_2	E
8	D	H	H	CH_2	E
9	A	Et	H	CH_2O	Z
10	A	H	H	CH_2O	Z
11	G	H	H	CH_2O	Z
12	G	^iPr	CH_2OH	CH_2O	E
13	G	H	CH_2OH	CH_2O	E
14	A	^iPr	CH_2OH	CH_2O	E
15	A	H	CH_2OH	CH_2O	E
16	A	^iPr	CH_2OH	CH_2	E
17	A	H	CH_2OH	CH_2	E
18	G	H	CH_2OH	CH_2	E

Example 1

(E)-9-(4-Phosphonobut-3-enyloxy)guanine

a) To a mixture of 2-[di-(t-butoxycarbonyl)]amino-9-hydroxy-6-methoxypurine (154mg, 404μmol), diisopropyl (E)-4-hydroxybut-1-enylphosphonate (89mg, 404μmol) and triphenylphosphine (159mg, 606μmol) in dry tetrahydrofuran (4ml) stirred at 0°C was added diethyl azodicarboxylate (105mg, 606μmol). The mixture was allowed to warm to room temperature and stirred for 2.3hr. The solvent was removed and the residue was purified by column chromatography on silica gel eluting with ethyl acetate-methanol (20:1) to give (E)-2-[di-(t-butoxycarbonyl)]amino-9-[4-(diisopropoxyphosphoryl)but-3-enyloxy]-6-methoxypurine as a colourless gum (150mg, 62%); λ_{max} (EtOH) 255 (12,300)nm; υ_{max} (KBr) 3440, 3220, 2975, 1790, 1600, 1370, 1280 and 1100cm^{-1}; δ_H[$(CD_3)_2SO$] 1.22 (12H, dd, J 6 Hz and 7 Hz, $CH(C\underline{H}_3)_2$), 1.40 (18H, S, $C(C\underline{H}_3)_3$), 2.70 (2H, m, CH_2), 4.08 (3H, s, CH_3O), 4.53 (4H, m, CH_2O and $C\underline{H}(CH_3)_2$), 5.98 (1H, dd, J 17 Hz and 19 Hz, PC$\underline{H}$=CH), 6.25 (1H, ddt, J 6 Hz, 17 Hz and 22 Hz, PCH=C$\underline{H}$), 8.71 (1H, s, 8-H) (Found: M^+ 599.2724. $C_{26}H_{42}N_5O_9P$ requires M^+ 599.2720).

b) To a solution of (E)-2-[di-(t-butoxycarbonyl)]-amino-9-[4-(diisopropoxyphosphoryl)but-3-enyloxy]-6-methoxypurine (106mg, 177μmol) in dichloromethane (5ml) was added bromotrimethylsilane (0.54g, 353μmol) and the mixture was stirred at room temperature under dry nitrogen for 18hr. The solution was evaporated to dryness and the residue azeotroped with methanol (x3). The residue was recrystallized from methanol-water (4:1) (10ml) to give

(E)-9-(4-phosphonobut-3-enyloxy)guanine as cream coloured crystals (45mg, 84%), m.p. >330°C; λ_{max} (EtOH) 255, 266nm; υ_{max} (KBr) 3200, 3120, 2740, 1760, 1690, 1635, 1470, 1235 and 1160cm^{-1}; δ_H[$(CD_3)_2SO$] 2.60 (2H, M, CH_2), 4.40 (2H, t, J 7 Hz, CH_2O) 5.92 (1H, dd, J 17 Hz and 19 Hz, PC<u>H</u>=CH), 6.50 (1H, ddt, J 6 Hz, 17 Hz and 22 Hz, PCH=C<u>H</u>), 6.60 (2H, br.s, NH_2), 7.90 (1H, s, H-8), 10.65 (1H, br.s, H-1); FABMS (thioglycerol) 302 (MH^+) (Found: C, 35.37; H, 4.01; N, 23.20%. $C_9H_{12}N_5O_5P.0.2H_2O$ requires C, 35.46; H, 4.10; N, 22.98%).

<u>Example 2</u>

<u>(E)-9-[4-(Diisopropoxyphosphoryl)but-3-enyloxy]adenine</u>

a) To a mixture of 9-hydroxy-6-phthalimidopurine (141mg, 500µmol), diisopropyl (<u>E</u>)-4-hydroxybut-1-enylphosphonate (110g, 500µmol) and triphenylphosphine (197mg, 750µmol) in tetrahydrofuran (5ml) stirred at 0°C was added diethyl azodicarboxylate (131mg, 750µmol). The mixture was then stirred at room temperature for 2hr. The solvent was removed and the residue purified by column chromatography on silica gel eluting with dichloromethane-methanol (49:1, 16:1) to give (<u>E</u>)-9-[4-(diisopropoxy-phosphoryl)but-3-enyloxy]-6-phthalimidopurine as a gum (200mg, 80%); λ_{max} (EtOH) 273 (14,380)nm; υ_{max} (film) 2970, 1730, 1590, 1570, 1355, 1240 and 975cm^{-1}; δ_H[$(CD_3)_2SO$] 1.24 (12H, pseudo t, J 6 Hz, $CH(C\underline{H}_3)_2$), 2.77 (2H, m, CH_2), 4.60 (2H, m, $C\underline{H}(CH_3)_2$), 4.66 (2H, t, J 6 Hz, CH_2O), 6.07 (1H, dd, J 17 Hz and 20 Hz, PC<u>H</u>=CH), 6.75 (1H, ddt, J 6 Hz, 17 Hz and 22 Hz, PCH=C<u>H</u>), 8.00-8.25 (4H, m, Ph), 9.00 (1H, s, H-2/H-8), 9.08 (1H, s, H-2/H-8) (Found: M^+ 499.1620. $C_{23}H_{26}N_5O_6P$ requires M^+ 499.1621).

b) A mixture of (E)-9-[4-(Diisopropoxyphosphoryl)but-3-enyloxy]-6-phthalimidopurine (186mg, 370μmol) and methylhydrazine (18mg, 390μmol) in ethanol (4ml) was stirred at room temperature for 1.5hr. The solvent was removed and the residue purified by column chromatography on silica gel eluting with dichloromethane-methanol (4:1) to afford (E)-9-[4-(diisopropoxyphosphoryl)but-3-enyloxy]-adenine as a gum (120mg, 88%); λ_{max} (EtOH) 260 (12,860)nm; υ_{max} (film) 3310, 3170, 2970, 1640, 1590, 1290, 1230 and 980cm^{-1}; δ_H[$(CD_3)_2SO$] 1.26 (12H, pseudo t, J 6 Hz, $CH(C\underline{H}_3)_2$), 2.67 (2H, m, CH_2), 4.50 (4H, m, CH_2O and $C\underline{H}(CH_3)_2$), 6.05 (1H, dd, J 17 Hz and 22 Hz, PC$\underline{H}$=CH), 6.70 (1H, ddt, J 6 Hz, 17 Hz and 22 Hz, PCH=C$\underline{H}$), 7.38 (2H, br.s, NH_2), 8.14 (1H, s, H-2/H-8), 8.36 (1H, s, H-2/H-8) (Found: M^+ 369.1568. $C_{15}H_{24}N_5O_4P$ requires M^+ 369.1566).

Example 3

(E)-9-(4-Phosphonobut-3-enyloxy)adenine

To a solution of (E)-9-[4-(diisopropoxyphosphoryl)but-3-enyloxy]adenine (105mg, 284μmol) in dichloromethane was added bromotrimethylsilane (0.87g, 5.68mmol). The resulting white suspension was stirred at room temperature under dry nitrogen for 18hr. The solution was evaporated to dryness and the residue azeotroped with methanol (x 3). The residue was purified by column chromatography on reverse phase silica gel eluting with water to give (E)-9-(4-phosphonobut-3-enyloxy)adenine as a white solid (68mg, 84%), m.p. 249-251°C; λ_{max} (MeOH) 260 (11,985)nm; υ_{max} (KBr) 3110, 2300, 1695, 1470, 1410, 1330 and 1030cm^{-1}; δ_H[$(CD_3)_2SO$] 2.62 (2H, m, CH_2), 4.50 (2H, t, J 7 Hz, CH_2O), 5.94 (1H, dd, J 17 Hz and 22 Hz, PC$\underline{H}$=CH), 6.50 (1H, ddt, J 6

Hz, 17 Hz and 22 Hz, PCH=CH), 7.39 (2H, br.s, NH_2), 8.16 (1H, s, H-2/H-8), 8.35 (1H, s, H-2/H-8); FABMS (thioglycerol) 286 (MH^+) (Found: C, 35.75; H, 4.00; N, 23.14; Br, 5.41%. $C_9H_{12}N_5O_4P.0.2HBr$ requires C, 35.86; H, 4.08; N, 23.24; Br, 5.30%).

Example 4

(E)-9-[4-(Diisopropoxyphosphoryl)but-3-enyl]adenine

a) To a mixture of 6-chloropurine (414mg, 2.67mmol), diisopropyl (E)-4-hydroxybut-1-enylphosphonate (630mg, 2.67mmol) and triphenyl phosphine (1.05g, 4.00mmol) in dry tetrahydrofuran (30ml) stirred at 0°C was added diethyl azodicarboxylate (0.70g, 4.02mmol). the mixture was allowed to warm to room temperature and stirred for 27.5hr. The solvent was removed and the residue was purified by column chromatography on silica gel eluting with dichloromethane-methanol (24:1, 13:1) to give (E)-6-chloro-9-[4-(diisopropoxyphosphoryl)but-3-enyl]purine as a white solid (0.37g, 37%), m.p. 105°C; λ_{max} (EtOH) 266 (9,260)nm; υ_{max} (KBr) 3435, 2980, 1590, 1560, 1330, 1230 and 1210cm^{-1}; $\delta_H(CDCl_3)$ 1.25 (12H, dd, J 6Hz and 21Hz, $CH(CH_3)_2$), 2.88 (2H, m, CH_2), 4.45 (2H, t, J 7Hz, CH_2N), 4.55 (2H, m, $CH(CH_3)_2$), 5.68 (1H, dd, J 17Hz and 20Hz, PCH=CH), 6.70 (1H, ddt, J 7Hz, 17Hz and 22Hz, PCH=CH), 8.10 (1H, s, H-2/H-8), 8.77 (1H, s, H-2/H-8); FABMS (thioglycerol) 373 (MH^+) (Found: C, 48.40; H, 6.03; N, 14.81%. $C_{15}H_{22}ClN_4O_3P$ requires C, 48.33; H, 5.95; N, 15.03%).

b) A solution of (E)-6-chloro-9-[4-(diisopropoxyphosphoryl)but-3-enyl]purine (309mg, 829mmol) in saturated ethanolic ammonia (35ml) was heated at 80°C in a stainless steel autoclave for 5hr. The solvent was removed and the

residue purified by column chromatography on silica gel eluting with ethyl acetate-methanol (3:1) to give (E)-9-[4-(diisopropoxyphosphoryl)but-3-enyl]adenine as a white solid (205mg, 70%), m.p. 121-122°C; λ_{max} (EtOH) 262 (11,855)nm; υ_{max} (KBr) 3320, 3175, 2935, 1650. 1600, 1575, 1475 and 1240cm^{-1}; δ_H($CDCl_3$) 1.25 (12H, dd, J 6Hz and 19Hz, $CH(C\underline{H}_3)_2$), 2.84 (2H, m, CH_2), 4.35 (2H, t, J 7HZ, CH_2N), 4.55 (2H, m, $C\underline{H}(CH_3)_2$), 5.69 (1H, dd, J 17Hz and 19Hz, $PC\underline{H}$=CH), 5.76 (2H, s, NH_2), 6.70 (1H, ddt, J 7Hz, 17Hz and 22Hz, PCH=C$\underline{H}$), 7.79 (1H, s, H-2/H-8), 8.37 (1H, s, H-2/H-8) (Found: MH^+ 354.1695. $C_{15}H_{24}N_5O_3P$ requires MH^+ 354.1695).

Example 5

(E)-9-[4-Phosphonobut-3-enyl]adenine

To a solution of (E)-9-[4-(diisopropoxyphosphoryl)-but-3-enyl]adenine (111mg, 314µmol) in dichloromethane (6ml) was added bromotrimethylsilane (0.9g, 6.28mmol) and the mixture was stirred at room temperature under dry nitrogen for 18hr. The solution was evaporated to dryness and the residue azeotroped with methanol (x3). The residue was purified by column chromatography on reverse phase silica gel eluting with water to give (E)-9-(4-phosphono-but-3-enyl)adenine as a white solid (69mg, 81%), m.p. 263-266°C; λ_{max} (MeOH) 261 (10,810)nm; υ_{max} (KBr) 3360, 3095, 1685, 1605, 1520, 1415, and 1228cm^{-1}; δ_H(D_2O + one drop of NH_4OH solution) 2.63 (2H, m, CH_2), 4.31 (2H, t, J 7Hz, CH_2N), 5.72 (1H, pseudo-t, J 17Hz, $PC\underline{H}$=CH), 6.14 (1H, pseudo-tt, J 7Hz and 17Hz, PCH=C$\underline{H}$), 8.13 (1H, s, H-2/H-8), 8.19 (1H, s, H-2/H-8); FABMS (thioglycerol) 270 (MH^+) (Found: C, 37.29; H, 4.38; N, 24.09%. $C_9H_{12}N_5O_3P.0.25HBr$ requires C, 37.35; H, 4.27; N, 24.20%).

Example 6

(E)-9-(4-Phosphonobut-3-enyl)guanine

a) To a mixture of 2-amino-6-chloropurine (0.6g, 3.81mmol), diisopropyl (E)-4-hydroxybut-1-enylphosphonate (0.90g, 3.81mmol) and triphenyl phosphine (2.00g, 7.62mmol) in dry N,N-dimethylformamide (30ml) stirred at 0°C under dry nitrogen was added diethyl azodicarboxylate (1.33g, 7.62mmol). The mixture was allowed to warm to room temperature and stirred for 1.3hr. The solvent was removed and the residue was purified by column chromatography on silica gel eluting with dichloromethane-methanol (9:1) to afford (E)-2-amino-6-chloro-9-[4-(diisopropoxyphosphoryl)but-3-enyl]purine as a light brown gum (0.52g, 35%), m.p. 150°C; λ_{max} (EtOH) 311 (6,760), 249 (5,420) and 224 (24,320)nm; υ_{max} (KBr) 3385, 3320, 3208, 1635, 1615, 1560, 1520, 1410 and 1240cm^{-1}; δ_H[$(CD_3)_2SO$] 1.08 (6H, d, J 6Hz, $CH(C\underline{H}_3)_2$), 1.16 (6H, d, J 6Hz, $CH(C\underline{H}_3)_2$), 2.77 (2H, m, CH_2), 4.27 (4H, m, CH_2N and $C\underline{H}(CH_3)_2$), 5.69 (1H, dd, J 17Hz and 21Hz, PC$\underline{H}$=CH), 6.52 (1H, ddt, J 6Hz, 17Hz and 22Hz PCH=C$\underline{H}$), 6.89 (2H, br.s, D_2O exchangeable, NH_2), 8.11 (1H, s, H-8) (Found: MH^+ 388.1288. $C_{15}H_{23}ClN_5O_3P$ requires MH^+ 388.1305).

b) To a suspension of (E)-2-amino-6-chloro-9-[4-(diisopropoxyphosphoryl)but-3-enyl]purine (168mg, 433µmol) in dichloromethane (8ml) stirred at room temperature under dry nitrogen was added bromotrimethylsilane (1.33g, 8.66mmol). The mixture was stirred for 18hr then evaporated to dryness. The residue was suspended in water (20ml), concentrated hydrochloric acid (3ml) added and the mixture heated at 100°C for 1.7hr. The solution was neutralized by addition of 2.5M sodium hydroxide solution then evaporated to dryness. The residue was purified by column

chromatography on reverse phase silica gel eluting with water to give (E)-9-[4-phosphonobut-3-enyl]guanine as a white solid (66mg, 53%), m.p. 290-294°C (decomp.); λ_{max} (MeOH) 257 (8,660)nm; υ_{max} (KBr) 3425, 3150, 2745, 1740, 1635, 1490, 1240 and 1190cm^{-1}; δ_H(D_2O + one drop of NH_4OH solution) 2.62 (2H, m, CH_2), 4.15 (2H, t, J 7Hz, CH_2N), 5.78 (1H, pseudo-t, J 17Hz, PC<u>H</u>=CH), 6.15 (1H, pseudo-tt, J 7Hz and 18Hz, PCH=C<u>H</u>), 7.80 (1H, s, H-8); FABMS (thioglycerol) 286 (MH+) (Found: C, 37.25; H, 4.10; N, 24.07%. $C_9H_{12}N_5O_4P.0.2H_2O$ requires C, 37.42; H, 4.32; N, 24.25%).

<u>Example 7</u>

<u>(E)-2,6-Diamino-9-[4-(diisopropoxyphosphoryl)but-3-enyl]purine</u>

A solution of (E)-2-amino-6-chloro-9-[4-(diisopropoxyphosphoryl)but-3-enyl]purine (370mg, 954µmol) in saturated ethanolic ammonia (60ml) was heated at 100°C in a stainless steel autoclave for 7hr. The solution was allowed to cool then the solvent was removed. The residue was purified by column chromatography on silica gel eluting with dichloromethane-methanol (19:1, 9:1) to give (E)-2,6-diamino-9-[4-(diisopropoxyphosphoryl)-but-3-enyl]purine as a white solid (175mg, 50%), m.p. 211-213°C; λ_{max} (MeOH) 256 (7,860) and 283 (9,670)nm; υ_{max} (KBr) 3460, 3325, 3174, 1630, 1590, 1470, 1410 and 1250cm^{-1}; δ_H[$(CD_3)_2SO$] 1.11 (6H, d, J 6Hz, $CH(C\underline{H}_3)_2$), 1.17 (6H, d, J 6Hz, $CH(C\underline{H}_3)_2$), 2.50 (2H, m, CH_2), 4.12 (2H, t, J 7Hz, CH_2N), 4.33 (2H, m, $C\underline{H}(CH_3)_2$), 5.73 (2H, s, D_2O exchangeable, NH_2), 5.73 (1H, dd, J 17Hz and 20Hz, PC<u>H</u>=CH), 6.55 (1H, ddt, J 7Hz, 17Hz and 20Hz, PCH=C<u>H</u>), 6.60 (2H, s, D_2O exchangeable, NH_2), 7.68 (1H, s, H-8) (Found: MH^+ 369.1803. $C_{15}H_{25}N_6O_3P$ requires MH^+ 369.1804).

Example 8

(E)-2,6-Diamino-9-(4-phosphonobut-3-enyl)purine

To a solution of (E)-2,6-diamino-9-[4-(diisopropoxy-phosphoryl)but-3-enyl]purine (144mg, 391μmol) in dichloromethane (10ml) was added bromotrimethylsilane (1.20g, 7.82mmol) and the mixture stirred at room temperature under dry nitrogen for 18hr. The resulting white suspension was evaporated to dryness and the residue azeotroped with methanol (x6). The residue was purified by column chromatography on reverse phase silica gel eluting with water to give a product which n.m.r. analysis showed to be the monoester. To a suspension of the monoester (approx. 306μmol) in dry N,N-dimethylformamide (10ml) was added bromotrimethylsilane (1.16g, 7.58mmol) and the resulting solution was stirred at room temperature under dry nitrogen for 18hr. The solution was evaporated to dryness and the residue azeotroped with methanol (x3) then acetone-water (1:1) (x3). The residue was purified by column chromatography on reverse phase silica gel eluting with water to give (E)-2,6-diamino-9-(4-phosphonobut-3-enyl)purine as a white solid (30mg, 27%), m.p. >325°C; λ_{max} (MeOH) 256 (6,570) and 285 (6,490)nm; υ_{max} (KBr) 3410, 1710, 1670, 1630, 1590, 1420, 1220 and 1135cm^{-1}; δ_H[$(CD_3)_2SO$ + one drop NH_4OH solution] 2.50 (2H, m, CH_2) 4.05 (2H, t, J 7Hz, CH_2N), 5.70 (1H, psuedo-t, J 17Hz, PC<u>H</u>=CH), 5.81 (2H, s, NH_2), 6.10 (1H, pseudo-tt, J 7Hz and 20Hz, PCH=C<u>H</u>), 6.69 (2H, s, NH_2), 7.76 (1H, s, H-8); FABMS (thioglycerol) 285 (MH^+).

Example 9

(Z)-9-[4-(Diethoxyphosphoryl)but-3-enyloxy]adenine

a) To a mixture of 9-hydroxy-6-phthalimidopurine (320mg, 1.14mmol), diethyl (Z)-4-hydroxybut-1-enylphosphonate

(250mg, 1.20mmol) and triphenyl phosphine (448mg, 1.71mmol) in dry tetrahydrofuran (11ml) stirred at 0°C under dry nitrogen was added diethyl azodicarboxylate (296mg, 170mmol). The mixture was allowed to warm to r.t. and stirred for 2.3hr. The solvent was removed and the residue was purified by column chromatography on silica gel eluting with acetone-hexane (1:1, 4:3) to give (Z)-9-[4-(diethoxyphosphoryl)but-3-enyloxy]-6-phthalimidopurine as a light brown gum (320mg, 60%); λ_{max} (EtOH) 271 (14,680)nm; υ_{max} (KBr) 2980, 1735, 1595, 1575, 1360, 1330, 1245 and 1025cm^{-1}; δ_H[$(CD_3)_2SO$] 1.23 (6H, t, J 7Hz, CH_3), 3.05 (2H, m, CH_2), 3.98 (4H, dq, J 7Hz and 8Hz, $C\underline{H}_2CH_3$), 4.63 (2H, t, J 6Hz, CH_2O), 5.90 (1H, dd, J 14Hz and 20Hz, PC$\underline{H}$=CH), 6.75 (1H, ddt, J 7Hz, 14Hz and 52Hz, PCH=C$\underline{H}$), 8.05 (4H, m, C_6H_4), 9.05 (1H, s, H-2/H-8), 9.10 (1H, s, H-2/H-8) (Found: C, 53.77; H, 4.87; N, 14.52%; MH^+ 472.1384. $C_{21}H_{22}N_5O_6P$ requires C, 53.50; H, 4.70; N, 14.86%; MH^+ 472.1386).

b) A mixture of (Z)-9-[4-(diethoxyphosphoryl)but-3-enyloxy]-6-phthalimidopurine (305mg, 647mmol) and methylhydrazine (31.3mg, 679µmol) in ethanol (7ml) was stirred at room temperature for 1.5hr. The solvent was removed and the residue was purified by column chromatography on silica gel eluting with dichloromethane-methanol (19:1, 9:1) to afford (Z)-9-[4-(diethoxyphosphoryl)but-3-enyloxy]adenine as a colourless gum (177mg, 80%); λ_{max} (EtOH) 260 (13,045)nm; υ_{max} (KBr) 3320, 3175, 2980, 1645, 1595, 1325, 1295 and 1240cm^{-1}; δ_H[$(CD_3)_2SO$] 1.22 (6H, t, J 7Hz, CH_3), 2.95 (2H, m, CH_2), 3.97 (4H, pseudo qu, J 7Hz, $C\underline{H}_2CH_3$), 4.46 (2H, t, CH_2O), 5.85 (1H, dd, J 14Hz and 20Hz, PC$\underline{H}$=CH), 6.65 (1H,

ddt, J 7Hz, 14Hz and 52Hz, PCH=CH), 7.38 (2H, br.s, NH_2), 8.15 (1H, s, H-2/H-8), 8.41 (1H, s, H-2/H-8) (Found: C, 45.30; H, 5.92; N, 20.17%; M^+ 341.1253. $C_{13}H_{20}N_5O_4P.0.3H_2O$ requires C, 45.03; H, 5.96; N, 20.20%; M^+ 341.1253).

Example 10

(Z)-9-(4-Phosphonobut-3-enyloxy)adenine

To a solution of (Z)-9-[4-(diethoxyphosphoryl)-but-3-enyloxy]adenine (145mg, 425μmol) in dichlormethane (10ml) was added bromotrimethylsilane (1.29g, 8.48mmol) and the resulting solution was stirred at room temperature under dry nitrogen for 18hr. The solvent was removed and the residue was azeotroped with methanol (x5). The residue was purified by column chromatography on reverse phase silica gel eluting with water to give (Z)-9-(4-phosphonobut-3-enyloxy]adenine as a white solid (98mg, 81%), m.p. 238°C; λ_{max} (MeOH) 260 (13,515)nm; υ_{max} (KBr) 3420, 3200, 3085, 2970, 1700, 1610, 1485, 1415 and 1335cm^{-1}; $\delta_H[(CD_3)_2SO]$ 2.94 (2H, m, CH_2), 4.43 (2H, t, J 7Hz, CH_2O), 5.77 (1H, dd, J 14Hz and 17Hz, PCH=CH), 6.40 (1H, ddt, J 7Hz, 14Hz and 47Hz, PCH=CH), 7.40 (2H, br.s, NH_2). 8.15 (1H, s, H-2/H-8), 8.42 (1H, s, H-2/H-8); FABMS (thioglycerol) 286 (NH^+) (Found: C, 36.71; H, 4.16; N, 23.96%. $C_9H_{12}N_5O_4P.0.4H_2O$ requires C, 36.96; H, 4.41; N, 23.95%).

Example 11

(Z)-9-(4-Phosphonobut-3-enyloxy)guanine

a) To a mixture of 2-[di-(t-butoxycarbonyl]-amino-9-hydroxy-6-methoxypurine (487mg, 1.28mmol), diethyl (Z)-4-hydroxybut-1-enylphosphonate (266mg, 1.28mmol) and triphenyl phosphine (504mg, 1.92mmol) in dry tetrahydrofuran

(15ml) stirred at 0°C under dry nitrogen was added diethyl azodicarboxylate (332mg, 1.91mmol). The mixture was allowed to warm to room temperature and stirred for 1.5hr. The solvent was removed and the residue was purified by column chromatography on silica gel eluting with acetone-hexane (1:1) to give (Z)-2-[di-t-butoxycarbonyl]amino-9-[4-(diethoxyphosphoryl)-but-3-enyloxy]-6-methoxypurine as a colourless gum (443mg, 61%); λ_{max} (EtOH) 256 (10,920)nm; υ_{max} (KBr) 2980, 2360, 1790, 1760, 1590, 1475, 1370, 1280 and 1255cm^{-1}; δ_H[$(CD_3)_2SO$] 1.21 (6H, t, J 7Hz, $CH_2C\underline{H}_3$), 1.40 (18H, s, $C(C\underline{H}_3)_3$), 2.97 (2H, m, CH_2), 3.95 (4H, pseudo qu, J 7Hz, $C\underline{H}_2CH_3$), 4.50 (2H, t, J 7Hz, CH_2O), 5.83 (1H, dd, J 14Hz and 19Hz, PC$\underline{H}$=CH), 6.68 (1H, ddt, J 7Hz, 14Hz and 52Hz, PCH=C$\underline{H}$), 8.75 (1H, s, H-8); CIMS (ammonia) 572 (MH^+) (Found: C, 50.16; H, 6.63; N, 12.63%. $C_{24}H_{35}N_5O_9P$ requires C, 50.43; H, 6.70; N, 12.25%).

b) To a solution of (Z)-2-[di-(t-butoxycarbonyl)]-amino-9-[4-(diethoxyphosphoryl)but-3-enyloxy]-6-methoxypurine (270mg, 472µmol) in dichloromethane (15ml) was added bromotrimethylsilane (1.44g, 9.44mmol) and the mixture was stirred at room temperature under dry nitrogen for 18hr. The solution was evaporated to dryness and the residue azeotroped with methanol (x1) then acetone-water (1:1) (x3). The residue was suspended in water and warmed on a steam bath. The mixture was cooled and purified by column chromatography on reverse phase silica gel eluting with water to afford (Z)-9-(4-phosphonobut-3-enyloxy)guanine as a white solid (60mg, 42%), m.p. 240-242°C; λ_{max} (MeOH) 255 (13,000)nm; υ_{max} (KBr) 3390, 3140, 1695, 1650, 1610, 1475, 1385 and 1165cm^{-1}; δ_H[$(CD_3)_2SO$] 2.91 (2H, m, CH_2), 4.32 (2H, t, J 7Hz, CH_2O), 5.75 (1H, dd, J 13Hz and 17Hz, PC$\underline{H}$=CH), 6.30 (1H, ddt, J 7Hz, 13Hz and 47Hz, PCH=C$\underline{H}$), 6.61 (2H, br.s, D_2O exchangeable, NH_2), 7.95 (1H, s, H-8), 10.63 (1H, br.s, D_2O exchangeable, H-1); FABMS (thioglycerol) 302 (MH^+) (Found:

C, 35.07; H, 3.79; N, 22.48%. $C_9H_{12}N_5O_5P.0.4H_2O$ requires C, 35.05; H, 4.18; N, 22.71%).

Example 12

(E)-9-[4-Diisopropoxyphosphoryl)-2-(hydroxymethyl)but-3-enyloxy]guanine

a) A mixture of 2-[di-(t-butoxycarbonyl)]amino-9-hydroxy-6-methoxypurine (0.62g, 1.6mmol), triphenylphosphine (0.43g, 1.6mmol), and diisopropyl (E)-3-(t-butyl diphenylsilyloxy)methyl-4-hydroxybut-1-enylphosphonate (0.6g, 1.2mmol) in anhydrous THF (15ml) at 0°C was treated dropwise, slowly, with diethyl azodicarboxylate (0.25g, 1.6mmol). After stirring overnight at room temperature, the solvent was removed in vacuo and the residue chromatographed on silica gel, eluting with acetone-hexane (1:2) to give (E)-9-[2-(t-butyldiphenylsilyloxy)methyl-4-(diisopropoxyphosphoryl)but-3-enyloxy]-2-[di-(t-butoxycarbonyl)]amino-6-methoxypurine as a gum (0.7g, 66%); υ_{max} (KBr) 2977, 2932, 2858, 1792, 1757, 1734, 1592, 1472, and 1427cm^{-1}; δ_H ($CDCl_3$) 1.06 [9H, s, $C(CH_3)_3$], 1.30 [12H, s, $2xCH(CH_3)_2$], 1.43 (18H, s, $2xC(CH_3)_3$], 2.92 (1H, m, CH), 3.85 (2H, m, CH_2), 4.15 (3H, s, OCH_3), 4.4-4.75 [4H, m, CH_2, $2xCH(CH_3)_2$], 5.91 (1H, ddd, J 1, 17 and 19Hz, PCH=CH), 6.77 (1H, ddd, J 8, 17 and 25Hz, PCH=H), 7.3-7.85 (11H, m, $2xC_6H_5$, H-8) (Found: C, 59.77; H, 7.52; N, 7.79%. $C_{43}H_{62}N_5O_{10}PSi$ requires C, 59.50; H, 7.20; N, 8.07%).

b) A solution of (E)-9-[2-(t-butyldiphenylsilyloxy) methyl-4-(diisopropoxyphosphoryl)but-3-enyloxy]-2-[di-(t-butoxycarbonyl)]amino-6-methoxypurine(0.45g, 0.5mmol) in ethanol (10ml) and 5M hydrochloric acid (1ml, 5mmol) was

heated under reflux for 4.5h. The solvent was removed in vacuo and the residue was chromatographed on silica gel eluting with chloroform-methanol (10:1) to give the title compound as a solid (0.16g, 74%); υ_{max} 3381, 3160, 2981, 2935, 2751, 1685, 1632, 1596, and 1472cm^{-1}; δ_H [$(CD_3)_2SO$] 1.22 [12H, m, 2x($C\underline{H}_3)_2CH$], 2.82 (1H, m, CH), 3.57 (2H, m, CH_2), 4.3-4.6 [4H, m, 2x($C\underline{H}_3)_2CH$ plus CH_2ON], 4.91 (1H, t, J 5Hz, D_2O exchangeable OH), 6.00 (1H, ddd, J 1, 17 and 18Hz, PC$\underline{H}$=CH), 6.65 (3H, m, D_2O exchangeable NH_2 plus PCH=C$\underline{H}$), 7.87 (1H, s, H-8), 10.69 (1H, s, D_2O exchangeable H-1).

Example 13

(E)-9-(2-Hydroxymethyl-4-phosphonobut-3-enyloxy)guanine

A solution of (E)-9-[4-(E)-diisopropoxyphosphoryl)-2-(hydroxymethyl)but-3-enyloxy]guanine (0.16g, 0.38mmol) in anhydrous N,N-dimethylformamide (4ml) under nitrogen at 0°C was treated with trimethylsilylbromide (0.76ml, 5.8mmol), and the solution stirred at room temperature for 18h. The solvent was removed in vacuo coevaporating several times with methanol and methanol-toluene mixtures. The resulting gum was purified by chromatography twice on C18 reverse phase silica gel to give (E)-9-(2-hydroxymethyl-4-phosphonobut-3-enyloxy)guanine as a solid (22mg, 17%), λ_{max} (H_2O) 253nm (12,277); υ_{max} (KBr) 3422, 3125, 2922, 2852, 2752, 1691, 1639, 1611, 1552, 1533, 1474, and 1451 cm^{-1}; δ_H [$(CD_3)_2SO$] 2.70 (1H, m, CH), 3.30 (>3H, br.s, D_2O exchangeable OH's, plus H_2O), 3.55 (2H, m, $C\underline{H}_2OH$), 4.35 (2H, m, CH_2ON), 5.95 (1H, dd, J_1=J_2=17.9Hz, PC$\underline{H}$=CH), 6.45 (1H, m, PCH=C$\underline{H}$), 6.60 (2H, br.s, D_2O exchangeable NH_2), 7.85 (1H, s, H-8), 10.63 (1H, br.s, H-1) (Found: C, 35.50; H, 4.27; N, 20.96% $C_{10}H_{14}N_5O_6P.0.4H_2O$ requires C, 35.49; H, 4.41; N, 20.69%).

Example 14

(E)-9-[4-(Diisopropoxyphosphoryl)-2-(hydroxymethyl)but-3-enyloxy]adenine

a) A mixture of 9-hydroxy-6-phthalimidopurine (0.94g, 3.4mmol), diisopropyl(E)-3-t-(butyldiphenylsilyloxy)methyl-4-hydroxybut-1-enylphosphonate (1.3g, 2.6mmol) and triphenylphosphine (0.88g, 3.4mmol) at 0°C in anhydrous THF (20ml) was treated dropwise, slowly with diethyl azodicarboxylate (0.53g, 3.4mmol) in anhydrous THF (5ml). After stirring overnight at room temperature, the solvent was removed and the residue chromatographed on silica gel eluting with ethyl acetate-hexane (1:1), increasing polarity to ethyl acetate, to give (E)-9-[2-(t-butyldiphenylsilyloxy)methyl-4-(diisopropoxyphosphoryl)but-3-enyloxy]-6-phthalimidopurine as a glass (1.22g, 62%); υ_{max} 3447, 3071, 2978, 2931, 2858, 1792, 1737, 1598, 1577, 1468, 1455, 1428, and 1406cm^{-1}; δ_H ($CDCl_3$) 1.07 [9H, s, $C(CH_3)_3$], 1.29 (3H, d, J 6Hz, CH_3CH), 1.30 (3H, d, J 6Hz, CH_3CH), 1.34 [6H, d, J 6Hz, $(CH_3)_2CH$], 3.0 (1H, s, CH), 3.90 (2H, m, CH_2), 4.70 (4H, m, CH_2, 2x$CH(CH_3)_2$], 6.00 (1H, ddd, J 1.4, 17.3 and 19.0Hz, PCH=CH), 6.81 (1H, ddd, J 7, 17, and 22Hz, PCH=CH), 7.3-8.2 (15H, m, C_6H_4, 2xC_6H_5, H-2/H-8), 9.04 (1H, s, H-2/H-8) (Found: C, 61.58; H, 6.09; N, 8.56%. $C_{40}H_{46}N_5O_7PSi$ requires C, 61.84; H, 6.10; N, 9.01%).

b) A solution of (E)-9-[2-(t-butyldiphenylsilyloxy)-methyl-4-(diisopropoxyphosphoryl)but-3-enyloxy]-6-phthalimidopurine (1.17g, 1.5mmol) in dichloromethane (25ml) at 0°C was treated dropwise with methyl hydrazine (0.12ml, 2.2mmol). After stirring at room temperature for 1h, the solvent was removed in vacuo and the residue was dissolved in acetone-hexane (1:1) (30ml). After filtration of the insoluble white solid, the solvent was removed in vacuo and the residue chromatographed on silica gel, eluting with

acetone-hexane (1:1) increasing polarity to (2:1) to give (E)-9-[2-(t-butyldiphenylsilyloxy)methyl-4-(diisopropoxyphosphoryl)but-3-enyloxy]adenine as a gum (0.72g, 74%); υ_{max} (KBr) 3325, 3175, 2978, 2931, 2858, 2230, 1641, 1593, 1471, 1427, and 1415cm^{-1}; δ_H ($CDCl_3$) 1.04 [9H, s, $C(CH_3)_3$], 1.27 (3H, d, J 6Hz, CH_3CH), 1.28 (3H, d, J 6Hz, CH_3CH), 1.33 [6H, d, J 6Hz, $(CH_3)_2CH$], 2.92 (1H, m, CH), 3.85 (2H, m, CH_2), 4.47-4.75 [4H, m, 2x$CH(CH_3)_2$, CH_2ON], 5.69 (2H, s, D_2O exchangeable NH_2), 5.96 (1H, ddd, J 1.4, 17.3 and 19.2Hz, PCH=CH), 6.78 (1H, ddd, J 7, 17 and 22Hz, PCH=CH), 7.3-7.75 (11H, m, 2xC_6H_5, H-2/H-8), 8.34 (1H, s, H-2/H-8) (Found: MH^+ 638.2909 $C_{32}H_{44}N_5O_5PSi$ requires MH^+ 638.2928).

c) A solution of (E)-9-[2-(t-butyldiphenylsilyloxy)-methyl-4-(diisopropoxyphosphoryl)but-3-enyloxy]adenine (0.27g, 0.4mmol) in 3% methanolic hydrogen chloride (5ml) was heated at 60°C for 5.5h. The solvent was removed in vacuo and the residue chromatographed on silica gel eluting with chloroform-methanol (20:1) increasing polarity to (10:1) to give the title compound as a glass (0.14g, 83%); υ_{max} 3391, 3204, 2980, 2934, 1689, 1642, 1599, 1468 and 1400cm^{-1}; δ_H [$(CD_3)_2SO$] 3.60 (>3H, m, CH_2, D_2O exchangeable OH), 4.55 [4H, m, 2x$CH(CH_3)_2$, CH_2ON], 6.07 (1H, ddd, J 1, 17 and 18 Hz, PCH=CH), 6.65 (1H, ddd, J 7, 17 and 23Hz, PCH=CH), 7.80 (2H, s, D_2O exchangeable NH_2), 8.23 (1H, s, H-2/H-8), 8.46 (1H, s, H-2/H-8H) (Found: C, 40.27; H, 5.62; N, 14.37%. $C_{16}H_{26}N_5O_5P.0.85CHCl_3$ requires C, 40.41; H, 5.40; N, 14.00%).

Example 15

(E)-9-(2-Hydroxymethyl-4-phosphonobut-3-enyloxy)adenine

A solution of (E)-9-[4-(diisopropoxyphosphoryl)-2-(hydroxymethyl)but-3-enyloxy]adenine (0.25g, 0.63mmol) in anhydrous N,N-dimethylformamide (5ml) under nitrogen was

treated with trimethylsilylbromide (1.24ml, 9.4mmol) at 0°C and the solution stirred for 18h at room temperature. The solvent was removed in vacuo coevaporating several times with methanol and toluene and the residue chromatographed on C18 reverse phase silica gel eluting with water to give (E)-9-(2-hydroxymethyl-4-phosphonobut-3-enyloxy)adenine as a solid (30g, 17%); λ_{max} (H_2O) 260nm (13711); υ_{max} (KBr) 3434, 1717, 1690, 1653, 1640, 1472, and 1414cm^{-1}; δ_H [$(CD_3)_2SO$] 2.78 (1H, m, CH), 3.38 (3H, br.s, 3xOH, H_2O), 3.60 (2H, m, CH_2OH), 4.48 (2H, m, CH_2ON), 5.99 (1H, m, PCH=CH), 6.48 (1H, m, PCH=CH), 7.37 (2H, br.s, D_2O exchangeable NH_2), 8.14 (1H, s, H-2/H-8), 8.34 (1H, s, H-2/H-8) (Found: C, 36.50; H, 4.45; N, 20.49%. $C_{10}H_{14}N_5O_5P.0.9H_2O$ requires C, 36.24; H, 4.77; N, 21.13%).

Example 16

(E)-9-[4-(Diisopropoxyphosphoryl)-2-(hydroxymethyl)but-3-enyl]adenine

a) To a solution of 6-chloropurine (213mg, 1.37mmol), diisopropyl (E)-3-(t-butyldiphenylsilyloxy)methyl-4-hydroxybut-1-enylphosphonate (694mg, 1.37mmol) and triphenyl phosphine (540mg, 2.06mmol) in N,N-dimethylformamide (22ml) stirred at 0°C under dry nitrogen was added diethyl azodicarboxylate (358mg, 2.06mmol). The solution was stirred at room temperature for 16h. The solvent was removed and the residue purified by column chromatography on silica gel eluting with acetone-hexane (1:4, 1:2) then ethyl acetate-methanol (99:1, 9:1) to give (E)-9-[2-(t-butyldiphenylsilyloxy)methyl-4-(diisopropoxyphosphoryl)but-3-enyl]-6-chloropurine as a colourless

gum (244mg, 28%); λ_{max} (EtOH) 265 (9,215)nm; υ_{max} (film) 2980, 2930, 1590, 1560, 1425, 1385, 1335 and 1245cm^{-1}; δ_H ($CDCl_3$) 1.10 (9H, s, CH_3), 1.20 (12H, m, $CH(C\underline{H}_3)_2$), 3.07 (1H, m, CH), 3.69 (2H, d, J 6Hz, CH_2O), 4.50 (4H, m, CH_2N and $C\underline{H}(CH_3)_2$), 5.57 (1H, pseudo-t, J 17Hz, $PC\underline{H}$=CH), 6.66 (1H, ddd, J 8Hz, 17Hz and 26Hz, PCH=$C\underline{H}$), 7.30-7.70 (10H, m, Ph), 8.00 (1H, s, H-2/H-8), 8.73 (1H, s, H-2/H-8) (Found: M^+ 641.2459. $C_{32}H_{42}N_4ClO_4PSi$ requires M^+ 641.2479).

b) A mixture of (E)-6-chloro-9-[2-(t-butyldiphenylsilyloxy)methyl-4-(diisopropoxyphosphoryl)but-3-enyl]-purine (244mg, 381µmol) and sodium azide (25mg, 381µmol) in N,N-dimethylformamide (7ml) was heated at 70°C for 2.8h. The solvent was removed and the residue purified by column chromatography on silica gel eluting with acetone-hexane (1:4, 1:1) to give (E)-6-azido-9-[2-(t-butyldiphenylsilyloxy)methyl-4-(diisopropoxyphosphoryl)but-3-enyl]purine as a gum (186mg, 75%); λ_{max} (EtOH) 282 (10,363)nm; υ_{max} (film) 2980, 2935, 2155, 1640, 1375, 1250 and 1110cm^{-1}; δ_H ($CDCl_3$) 1.00-1.40 (21H, m, CH_3), 3.05 (1H, m, CH), 3.70 (2H, m, CH_2O), 4.30-4.80 (4H, m, CH_2N and $C\underline{H}(CH_3)$), 5.58 (1H, m, $PC\underline{H}$=CH), 6.70 (1H, m, PCH=$C\underline{H}$), 7.30-7.70 (10H, m, C_6H_5), 7.86 (0.35H, s, H-2/H-8), 8.05 (0.65H, s, H-2/H-8), 8.64 (0.35H, s, H-2/H-8), 9.44 (0.65H, s, H-2/H-8)*; FABMS (TDE/Na) 670 (MNa^+), 648 (MH^+).

* Mixture of azido and tetrazolo tautomers

c) A solution of (E)-6-azido-9-[2-(t-butyldiphenylsilyloxy)methyl-4-(diisopropoxyphosphoryl)but-3-enyl]purine (320mg, 494µmol) and triphenylphosphine (194mg, 741µmol) in tetrahydrofuran (15ml) was stirred at room temperature for 21h. The solution was heated to 70°C and 5M hydrochloric acid (258µl, 1.29mmol) added. After 2h, the

solvent was removed and the residue was dissolved in 3% methanolic hydrogen chloride (10ml) and the solution stirred at room temperature for 2h. The solvent was removed, the residue dissolved in water and the solution neutralised by addition of aqueous sodium bicarbonate solution. The solution was evaporated to dryness and the residue purified by column chromatography on silica gel eluting with dichloromethane-methanol (9:1, 6:1) to give the title compound as a white solid (124mg, 63%), m.p. 130°C; λ_{max} (EtOH) 261 (13,074)nm; υ_{max} (KBr) 3325, 2980, 1645, 1600, 1475, 1420, 1240 and 990cm^{-1}; δ_H [$(CD_3)_2SO$] 1.10 (12H, m, CH_3), 3.07 (1H, m, CH), 3.50 (2H, t, J 5Hz, CH_2O), 4.10 (1H, m, C<u>H</u>$(CH_3)_2$)), 4.27 (3H, m, CH_2N and C<u>H</u>$(CH_3)_2$), 4.99 (1H, t, J 5Hz, D_2O exchangeable, OH), 5.59 (1H, dd, J 17Hz and 21Hz, PC<u>H</u>=CH), 6.52 (1H, ddd, J 8Hz, 17Hz and 22Hz, PCH=C<u>H</u>), 7.16 (2H, br.s, D_2O exchangeable, NH_2), 8.07 (1H, s, H-2/H-8), 8.12 (1H, s, H-2/H-8); CIMS 384 (MH^+) (Found: C, 48.92; H, 6.90; N, 17.58%. $C_{16}H_{26}N_5O_4P.0.5\ H_2O$ requires C, 48.97; H, 6.94; N, 17.85%).

Example 17

(E)-9-(2-Hydroxymethyl-4-phosphonobut-3-enyl)adenine

A solution of (<u>E</u>)-9-[2-hydroxymethyl-4-(diisopropoxyphosphoryl)but-3-enyl]adenine (107mg, 280µmol) and bromotrimethylsilane (0.86g, 5.61mmol) in <u>N</u>,<u>N</u>-dimethylformamide (5ml) was stirred at room temperature under dry nitrogen for 18h. The solvent was removed and the residue azetroped with methanol (x3). The residue was purified by column chromatography on reverse phase silica gel eluting with water to give (<u>E</u>)-9-(2-hydroxymethyl-4-phosphonobut-3-enyl)adenine as a white solid (34mg, 40%), m.p. >300°C; λ_{max} (MeOH) 262 (10,704)nm; υ_{max} (KBr) 3435, 1695, 1640, 1415, 1263, 1229 and 1030 cm^{-1}; δ_H [$(CD_3)_2SO/D_2O$] 2.73 (1H, m,

CH), 3.37 (2H, d, J 6Hz, CH_2O), 4.13 (1H, dd, J 7Hz and 14Hz, CH_2N), 4.30 (1H, dd, J 7Hz and 14Hz, CH_2N), 5.65 (1H, pseudo-t, J 17Hz, PC<u>H</u>=CH), 6.06 (1H, ddd, J 8Hz, 19Hz, PCH=C<u>H</u>), 8.10 (1H, s, H-2/H-8), 8.17 (1H, s, H-2/H-8); FABMS (thioglycerol) 300 (MH^+) (Found: C, 37.83; H, 4.83; N, 21.75%. $C_{10}H_{14}N_5O_4P.H_2O$ requires C, 37.86; H, 5.08; N, 22.07%).

<u>Example 18</u>

<u>(E)-9-(2-Hydroxymethyl-4-phosphonobut-3-enyl)guanine</u>

a) To a solution of 2-acetamido-6-chloropurine[1] (326mg, 1.54mmol) diisopropyl (<u>E</u>)-3-(<u>t</u>-butyldiphenylsilyloxy)-methyl-4-hydroxybut-1-enylphosphonate (775mg, 1.54mmol) and triphenyl phosphine (606mg, 2.31mmol) in <u>N</u>,<u>N</u>-dimethylformamide, stirred at 0°C under dry nitrogen, was added diethyl azodicarboxylate (0.40g, 2.31mmol). The solution was stirred at room temperature for 16h. The solvent was removed and the residue purified by column chromatography on silica gel eluting with ethyl acetate then ethyl acetate-methanol (19:1) to give (<u>E</u>)-2-acetamido-9-[2-(<u>t</u>-butyldiphenylsilyloxy)methyl-4-(diisopropoxyphosphoryl)-but-3-enyl]-6-chloropurine as a colourless gum (390mg, 36%); λ_{max} (EtOH) 224 (29,735), 260 (8,593) and 289 (9,915)nm; υ_{max} (KBr) 2980, 2930, 1695, 1610, 1575, 1515, 1375, 1285 and 1235cm^{-1}; δ_H ($CDCl_3$) 1.09 (9H, s, $C(CH_3)_3$), 2.53 (3H, s, $NCOCH_3$), 3.00 (1H, m, CH), 3.67 (2H, m, CH_2O), 4.25-4.60 (4H, m, CH_2N and C<u>H</u>$(CH_3)_2$), 5.58 (1H, pseudo-t, J 18Hz, PC<u>H</u>=CH), 6.67 (1H, ddd, J8Hz, 17Hz and 22Hz, PCH=C<u>H</u>), 7.50 (10H, m, C_6H_5), 7.86 (1H, s, H-8), 8.29 (1H, br.s, D_2O exchangeable, NH) (Found: M^+ 698.2697. $C_{34}H_{45}N_5O_5ClPSi$ requires M^+ 698.2695).

1. W.A. Bowles <u>et al.</u>, <u>J. Med. Chem.</u>, 1963, <u>6</u>, 471.

b) A solution of (E)-2-acetamido-9-[2-(t-butyldiphenylsilyloxy)methyl-4-(diisopropoxyphosphoryl)but-3-enyl]-6-chloropurine (330mg, 473μmol) in 7% methanolic hydrogen chloride (15ml) was stirred at room temperature for 7h. The solution was reduced to 1/3 volume then neutralised by addition of saturated sodium bicarbonate solution. The solvent was removed and the residue purified by column chromatography on silica gel eluting with dichloromethane-methanol (19:1, 6:1) to give (E)-2-amino-9-[4-(diisopropoxyphosphoryl)-2-(hydroxymethyl)but-3-enyl]-6-methoxypurine as a colourless gum (140mg, 72%); λ_{max} (EtOH) 249 (8,632) and 283 (9,086)nm; υ_{max} (KBr) 3335, 2980, 1610, 1585, 1475, 1410, 1400 and 1250cm^{-1}; δ_H [$(CD_2)_2SO$] 1.10 (12H, m, $CH(C\underline{H}_3)_2$), 3.02 (1H, m, CH), 3.50 (2H, t, J 5Hz, CH_2O), 3.94 (3H, s, CH_3O), 4.10-4.40 (4H, m, CH_2N and $C\underline{H}(CH_3)_2$), 4.97 (1H, t, J 5Hz, D_2O exchangeable, OH), 5.61 (1H, dd, J 17Hz and 20Hz, $PC\underline{H}$=CH), 6.43 (2H, br.s, D_2O exchangeable, NH_2), 6.50 (1H, ddd, J 8Hz, 17Hz and 22Hz, PCH=C$\underline{H}$), 7.80 (1H, s, H-8) (Found: MH^+ 414.1900. $C_{17}H_{28}N_5O_5P$ requires MH^+ 414.1906).

c) A solution of (E)-2-amino-9-[4-(diisopropoxyphosphoryl)-2-(hydroxymethyl)but-3-enyl]-6-methoxypurine (135mg, 327μmol) and bromotrimethylsilane (1.0g, 6.53mmol) in N,N-dimethylformamide (5ml) was stirred at room temperature under dry nitrogen for 18h. The solvent was removed and the residue azeotroped with methanol (x3). The residue was purified by column chromatography on reverse phase silica gel eluting with water to give the title compound as a white solid (42mg, 40%), m.p. >300°C; λ_{max} (MeOH) 256 (7,402)nm; υ_{max} (KBr) 3425, 1715, 1640, 1610, 1480, 1410, 1380 and 1160cm^{-1}; δ_H [$(CD_3)_2SO$] 2.80 (1H, m, CH), 3.40 (2H, d, J 5Hz, CH_2O), 4.04 (2H, m, CH_2N), 5.73 (1H, pseudo-t, H 18Hz, $PC\underline{H}$=CH), 6.35 (1H, ddd, J 7Hz, 17Hz and 22Hz, PCH=C$\underline{H}$), 6.50 (2H, br.s, D_2O exchangeable, NH_2),

7.60 (1H, s, H-8), 10.56 (1H, br.s, D_2O exchangeable, H-1); FABMS (thioglycerol) 316 (MH^+) (Found: C, 38.20; H, 4.82; N, 21.58%. $C_{10}H_{14}N_5O_5P.0.2H_2O.0.2$ DMF requires C, 38.19; H, 4.77; N, 21.84%).

5

Antiviral Activity

1. Plaque Reduction Test for Herpes Simplex Viruses 1 and 2

MRC-5 cells were grown to confluence in 24 well multi-dishes (well diameter = 1.5cm). The drained cell monolayers were each infected with approximately 50 infectious particles of herpes simplex virus 1 (HSV-1; strain SC16) or herpes simplex virus 2 (HSV-2; strain MS) in 100µl of phosphate-buffered saline. The virus was adsorbed for 1 hour at room temperature. After adsorption, residual inoculum was removed from each well and replaced with 0.5ml of Eagle's MEM containing 5% newborn calf serum and 0.9% agarose (A37). Once the agarose had set, dilutions of the test compound, which had been prepared in Eagle's MEM (containing 5% newborn calf serum), were added, each well receiving 0.5ml of liquid overlay. The test compound was diluted to give the following series of concentrations: 200, 60, 20, 6....0.06µg/ml; final concentrations in the assay ranged, therefore, between 100µg/ml and 0.03µg/ml. The infected cultures wereincubated at 37°C in a humidified atmosphere of 5% CO_2 until plaques were clearly visible (usually 1 day).

2. Plaque Reduction Test for Varicella-Zoster Virus

MRC-5 cells were grown to confluence in 24 well multi-dishes (well diameter = 1.5cm). The drained cell monolayers were each infected with approximately 50 infectious particles of varicella zoster virus (VZV; Ellen strain) in 100µl of phosphate-buffered saline. The virus was adsorbed for 1 hour at room temperature. After adsorption, residual inoculum was removed from each well and replaced with 0.5ml of Eagle's MEM containing 5% heat-inactivated foetal calf serum and 0.9% agarose (A37). Once the agarose had set, dilutions of the test compound, which had been prepared in

Eagle's MEM (containing 5% heat-inactivated foetal calf serum), were added, each well receiving 0.5ml of liquid overlay. The test compound was diluted to give the following series of concentrations: 200, 60, 20, 6....0.06µg/ml; final concentrations in the assay ranged, therefore, between 100µg/ml and 0.03µg/ml. The infected cultures were incubated at 37°C in a humidified atmosphere of 5% CO_2 until plaques were clearly visible (5 or 6 days).

Cultures from 1 and 2 were fixed in formal saline, the agarose overlays were carefully washed off, and then the cell monolayers were stained with carbol fuchsin. A stereo microscope was used to count plaques. The IC_{50} (concentration of drug which inhibits the number of plaques formed by 50% relative to the number of plaques observed in virus control monolayers) of the test compound was calculated. In addition, the monolayers were examined for evidence of drug-induced cytotoxicity; the minimum concentration at which cytotoxicity occurs was recorded.

3. Plaque Reduction Test for Cytomegalovirus

MRC-5 cells were grown to confluence in 24 well multi-dishes (well diameter = 1.5cm). The drained cell monolayers were each infected with approximately 50 infectious particles of cytomegalovirus (CMV; AD-169 strain) in 100µl of phosphate-buffered saline. The virus wasadsorbed for 1 hour at room temperature. After adsorption, residual inoculum was removed from each well and replaced with 1ml of Eagle's MEM containing 10% heatinactivated foetal calf serum and 0.9% agarose (A37). Once the agarose had set, dilutions of the test compound, which had been prepared in Eagle's MEM (containing 10% heat-inactivated calf serum), were added, each well receiving 1ml of liquid overlay. The test compound was diluted to give the following series of concentrations: 200, 60, 20, 6....0.06µg/ml; final

concentrations in the assay range, therefore, between 100µg/ml and 0.03µg/ml. The infected cultures were incubated at 37°C in a humidified atmosphere containing 5% CO_2 until plaques were clearly visible (about 12days). The cultures are fixed in formol saline, the agarose overlays were carefully washed off, and then the cell monolayers were stained with carbol fuchsin. A stereo microscope was used to count plaques. The IC_{50} (concentration of drug which inhibits the number of plaques formed by 50% relative to the number of plaques observed in virus control monolayers) of the test compound was calculated. In addition, the monolayers were examined for evidence of drug-induced cytotoxicity; the minimum concentration at which cytotoxicity occurs was recorded.

4. <u>CPE Inhibition Test (Established Monolayer) for Lentiviruses</u>

3×10^4 sheep choroid plexus (SCP) cells were plated into individual wells of a 96 well microtitre plate in 100µl of Eagle's MEM with Hanks' salts containing 10% heat inactivated foetal calf serum (FCS). When monolayers had become established (after 1 or 2 days growth) they were washed with 200µl of maintenance medium (Eagle's MEM with Hanks' salts containing 0.5% FCS) and infected with 100µl of visna virus (strain K184) in maintenance medium (30 $TCID_{50}$/ml). Test samples were diluted with maintenance medium in further 96 well microtitre plates over the range 200-0.06µg/ml by 3-fold dilution steps. 100µl of the diluted samples was then transferred directly onto virus-infected monolayers (final concentration range therefore 100-0.03µg/ml) and incubated at in a humidified atmosphere containing 5% CO_2 until virus-induced CPE was maximal in the untreated virus-infected controls (usually 12-14 days). The plates were fixed with formal saline and stained with crystal violet. Virus-induced CPE was then

scored microscopically and the minimum concentration of sample giving complete protection of the cell monolayers (MIC) determined.

5. Test for Human Immunodeficiency Virus (HIV)

a) Cell cytotoxicity test

Peripheral human lymphocytes were isolated by density gradient centrifugation from blood donations of healthy volunteers. The 'buffy coat'-fractions of these donations were provided by blood donation centres.

The buffy coat was diluted 1:1 with sterile phosphate buffered saline (PBS; 50 mM sodium phosphate, pH 7.4, 0,9% sodium chloride) and subsequently layered over Ficoll. Following centrifugation (30 minutes at 400 x g) the supernatant was discarded and the interphase containing the lymphocytes was recovered. The lymphocytes were washed two times in PBS and were resuspended finally in cell culture medium.

A viability staining was performed by means of the trypan blue dye-exclusion method. The concentration of cells in the suspension and the percentage of viable cells were calculated. Subsequently, the cell suspension was adjusted to a concentration of $1x10^7$ cells/ml. This cell suspension was transferred to tissue culture flasks: Two thirds of the cell suspension were polyclonally activated by addition of phytohemagglutinin (final concentration 5 µg.ml). One third of the cell suspension remained unstimulated.

The lymphocytes were cultivated in an incubator with a humidified atmosphere and 5% CO_2 for 48 to 64 hours at 37°C. Following this incubation period, cells were harvested by centrifugation, resuspended in cell culture medium and

counted. Stimulated and unstimulated cells were combined in a ratio of 2:1 and adjusted to a concentration of $2x10^6$ cells/ml with cell culture medium that contained, in addition, 10 units/ml of human recombinant interleukin-2.

Only those preparations of lymphocytes were employed for the screening test in which more than 70% of the stimulated lymphocytes expressed the CD 25 antigen and more than 45% of the lymphocytes expressed the CD 4 antigen.

100µg of this lymphocyte suspension was added to each well of microtiter plates containing the test compounds serially diluted over the range 100 µM to 0.1µM. Subsequently, the microtiter plates were cultivated for 4 days at 37°C.

Survival and proliferation of the lymphocytes grown in the presence of the compound were measured by a quantitative colorimetric assay. Viable cells cultivated in the presence of the dye MTT (3(4,5 Dimethylthiazol-2-yl)-3,5-diphenyltetrazolium) reduce this pale yellow substrate by activity of their mitochondrial dehydrogenases to a purple formazan. The amount of product which is a function of cell number and metabolic cellular activity was quantified photometrically. By this assay, potential cytotoxic and cytostatic effects of compounds towards lymphocytes were detected precisely.

Microtiter plates were centrifuged for 5 minutes at 900 x g. The supernatant was discarded and the cells of each well were resuspended in 50 µl of cell culture medium containing 2mg/ml of MTT. After four hours of incubation at 37°C 100 µl of solvent (isopropanol with 0,04 N HCl and 10% (v/v) Triton 100) was added to each well. By shaking the microtiter plates the formazan was solubilized.

Subsequently, the plates were evaluated in an ELISA photometer in the dual wavelength mode (measuring wavelength: 550 nm; reference wavelength: 690 nm).

For each well the difference in absorption (abs. 550 nm - abs. 690 nm) was calculated. These data provided the basis for further evaluation of the cytotoxicity test. The approximate CD_{50} -halfmaximal cytotoxic dose- of each compound was calculated.

b) HIV Suppression test

Peripheral human lymphocytes were prepared, cultivated, and harvested as above. Following the determination of the lymphocyte surface markers, unstimulated and mitogen stimulated cells were combined in a ratio of 1:2.

Under safety conditions these cells are infected with a standard preparation of HIV. The cells are sedimented by centrifugation. The supernatant was discarded and cells were resuspended in the HIV inoculum.

This inoculum is a liquid suspension of HIV-1 strain virus, pretested and adjusted to a titer that results in a synthesis of viral core protein p24 of >100 ng/ml at day four following infection of human lymphocytes according to the protocol.

$3x10^8$ lymphocytes were resuspended in 1 ml HIV inoculum and incubated at 37°C for 60 minutes. Subsequently, the cells were washed two times with 50 ml of culture medium and resuspended in culture medium containing 10 units/ml of human recombinant interleukin-2 to yield a cell concentration of $2x10^6$ cells/ml. 100µl of this cell suspension was added to each well of the microtiter plates

containing the diluted solutions of the compounds. The microtiter plates were cultivated in an incubator with a humidified atmosphere and 5% CO_2 at 37°C.

Accordingly, a fraction of lymphocytes was mock-infected with the same virus preparation that was heat inactivated (30 minutes at 56°C) prior to infection.

On each of the days 2,3 and 4 post infection one of the microtiter plates which had been established in triplicate was prepared for determination of viral replication. Viral RNA is determined within the cells whereas the viral core protein p24 was detected in the supernatant of the lymphocyte culture.

Accordingly, 150 µl of supernatant were removed from each well and transferred to the well of a microtiter plate containing 50 µl/well of SDS (sodium dodecylsulfate, 0.08%). These plates were stored frozen. 50 µl of stop solution (1% SDS, 20mM sodium acetate, pH 5.0, and 200 µg/ml heparin) were added to the cells remaining in each well. The plates were stored frozen.

The concentration of p24 synthesized by the HIV infected cells was determined by means of a sandwich ELISA, while the concentration of viral RNA was quantitated by nucleic acid hybridisation, using a ^{32}P-labelled DNA probe for the gag/pol region of the viral genome. Absolute levels of viral antigen and RNA in drug treated samples were compared with untreated, virus-infected controls and the percentage inhibition calculated.

6. Test for Feline Immunodeficiency virus (FIV)

Drug stocks were diluted to the appropriate concentration, in medium (eg 10mg/ml to 200 µg/ml). 150µl of each drug was dispersed in triplicate, across the top row of a microtitre plate (150µl of media for virus and cell control, vc and cc). 100µl of medium was dispersed into all other wells. The wells were serially diluted moving down the plate, removing 50µl from each well and transferring to the next row. 50µl was discarded from the bottom wells.

Trypsinisation was carried out from a confluent cell monolayer of Crandell Feline Kidney cells, in 10% trypsin, followed by resuspension in media at $1x10^6$ per ml, ensuring that a single cell suspension is achieved. (Media: 90% 1x RPMI, 25mM hepes buffer; 10% Foetal calf serum; 2% Glutamine; 2% penecillin/streptomycin.)

FIV Glasgow 8 virus was diluted to 4x the required virus challenge in medium (40 $TCID_{50}$/ml). The virus infected medium was mixed with an equal volume of cell suspension, 100µl of which was aliquoted into each well of the drug plate, except cell control. 100µl of cell suspension at $5x10^4$ per ml was added to the latter. This gives a final cell concentration of $2.5x10^4$ per ml, and drug range 100-0.03 µg/ml. The plates were incubated at 37°C, 5% CO_2 in an air humidified incabator for 11-14 days. The cells were fixed by immersing the plates in formol saline (10% formaldehyde; 10% 1.5M NaCl; 80% water) for 1 hour minimum. The cells were stained with 10% crystal violet for 15 minutes.

The assay was scored by looking for presence of syncitia and, virus induced cytopathic effect in the cell monolayers, under a microscope. Results are given as the minimum concentration of drug inhibiting syncitial production, minimum inhibitory concentration, MIC.

The results of the tests 1 to 6 were as follows:-

Antiviral Activity Against Herpesviruses

IC_{50} (µg/ml)

Example No.	HSV-1	HSV-2	VZV	CMV
1	<3	<3	<3	0.16
6	100	26	55	15
11	100	>100	>100	55
13	100	60	5	15
17	20	29	<3	11

No cytotoxicity for the cell monolayers was noted with concentrations of the compounds up to 100µg/ml in the HSV-1, HSV-2 and VZV tests. In the CMV test cytotoxicity was noted at concentrations of 10µg/ml, for example 1.

Antiviral Activity Against Visna Virus

Example No.	MIC (µg/ml)
1	<0.003
3	10
5	1
6	0.1
8	0.3
10	100
11	<0.03

At concentrations up to 100µg/ml, the compounds were not toxic for the SCP cell monolayers used in the tests.

Antiviral Activity Against HIV

Example No.	Concn. (μM)	% Inhibition on Day 4 after infection: Viral Antigen	Viral RNA
1	0.1	93	93
3	10	47	44
8	10	37	62
11	10	3	33

Slight toxicity (18% inhibition) at 0.1μM was noted for the compound of Example 1, although the CD_{50} was 1μM.

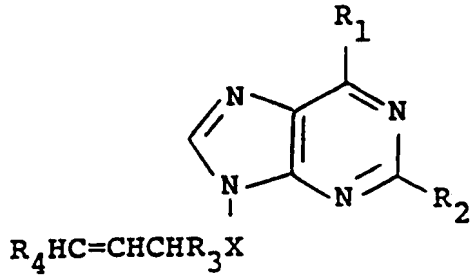
Antiviral Activity against FIV

Example No.	MIC (μg/ml)
1	0.01
3	10
13	0.10
15	30
17	0.03

No cytotoxicity for the cell monolayers was noted with concentrations up to 100 μg/ml for examples 1, 3 and 15. Cytotoxicity was noted at a concentration of 10μg/ml for example 17 and at 1μg/ml for example 13.

Claims

1. A compound of formula (I), or a pharmaceutically acceptable salt thereof:



(I)

wherein

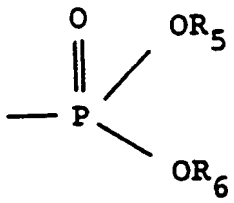
X is $-CH_2O$ or $-CH_2$;

R_1 is hydroxy or amino;

R_2 is hydrogen or amino;

R_3 is hydrogen, hydroxymethyl or acyloxymethyl; and

R_4 is a group of formula:



wherein

R_5 and R_6 are independently selected from hydrogen, C_{1-6} alkyl and optionally substituted phenyl.

2. A compound according to claim 1 wherein R_1 is hydroxy and R_2 is amino.

3. A compound according to claim 1 wherein R_1 is amino and R_2 is hydrogen.

4. A compound according to any one of claims 1 to 3 wherein R_3 is hydroxymethyl.

5. A compound according to any one of claims 1 to 4 wherein R_5 and R_6 are both hydrogen.

6. (E)-9-(4-Phosphonobut-3-enyloxy)guanine.

7. (E)-9-[4-(Diisopropoxyphosphoryl)but-3-enyloxy]-adenine.

8. (E)-9-(4-Phosphonobut-3-enyloxy)adenine.

9. (E)-9-[4-(Diisopropoxyphosphoryl)but-3-enyl]adenine.

10. (E)-9-[4-Phosphonobut-3-enyl]adenine.

11. (E)-9-(4-Phosphonobut-3-enyl)guanine.

12. (E)-2,6-Diamino-9-[4-(diisopropoxyphosphoryl)but-3-enyl]purine.

13. (E)-2,6-Diamino-9-(4-phosphonobut-3-enyl)purine.

14. (Z)-9-[4-(Diethoxyphosphoryl)but-3-enyloxy]adenine.

15. (Z)-9-(4-Phosphonobut-3-enyloxy)adenine.

16. (Z)-9-(4-Phosphonobut-3-enyloxy)guanine.

17. (E)-9-[4-Diisopropoxyphosphoryl)-2-(hydroxymethyl)-but-3-enyloxy]guanine.

18. (E)-9-(2-Hydroxymethyl-4-phosphonobut-3-enyloxy)-guanine.

19. (E)-9-[4-(Diisopropoxyphosphoryl)-2-(hydroxymethyl)-but-3-enyloxy]adenine.

20. (E)-9-(2-Hydroxymethyl-4-phosphonobut-3-enyloxy)-adenine.

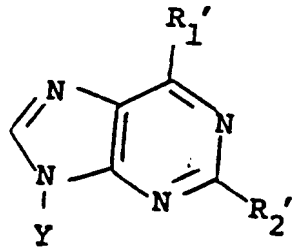
21. (E)-9-[4-(Diisopropoxyphosphoryl)-2-(hydroxymethyl)-but-3-enyl]adenine.

22. (E)-9-(2-Hydroxymethyl-4-phosphonobut-3-enyl)adenine.

23. (E)-9-(2-Hydroxymethyl-4-phosphonobut-3-enyl)guanine.

24. A compound according to claim 1 substantially as hereinbefore described with reference to the Examples.

25. A process for the preparation of a compound of formula (I), or a pharmaceutically acceptable salt thereof, which process comprises condensing a compound of formula (II):



(II)

with a side chain intermediate of formula (III):

$$R_4'HC=CHCHR_3'CH_2OH \qquad (III)$$

wherein, when X is $-CH_2O$ in formula (I), Y is OH and, when X is $-CH_2$, Y is H; R_1', R_2', R_3' and R_4' are R_1, R_2, R_3 and R_4 respectively, or groups or atoms convertible thereto; and thereafter, when desired or necessary, converting R_1', R_2', R_3' and/or R_4', when other than R_1, R_2, R_3 and/or R_4 to R_1, R_2, R_3 and/or R_4 respectively, and/or converting R_1', R_2', R_3' and/or R_4' when R_1, R_2, R_3 and/or R_4, to other R_1, R_2, R_3 and/or R_4, and/or forming a pharmaceutically acceptable salt thereof.

26. A pharmaceutical composition comprising a compound according to any one of claims 1 to 24, and a pharmaceutically acceptable carrier.

27. A compound according to any one of claims 1 to 24 for use as an active therapeutic substance.

28. A compound according to any one of claims 1 to 24 for use in treating viral infections or in treating neoplastic diseases.

29. Use of a compound according to any one of claims 1 to 24 in the manufacture of a medicament for use in the treatment of viral infections or neoplastic diseases.

30. A method of treatment of viral infections or neoplastic diseases in mammals, which comprises the administration to mammal in need of such treatment, an effective amount of a compound according to any one of claims 1 to 24.

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 91/01171

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl.5 C 07 F 9/6561 A 61 K 31/675

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl.5	C 07 F 9/00 A 61 K 31/00

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
A	EP,A,0343133 (MEDIVIR AKTIEBOLAG) 23 November 1989, see claims, (cited in the application)	1,26-30
A	Collection of Czechoslovak Chem. Commun., Vol. 53, no. 11B,November 1988, I. Rosenberg et al.:" Phosphonylmethoxyalkyl and phosphonylalkyl derivatives of adenine", pages 2753-2777, see page 2762, scheme 4, and pages 2774-2775, (cited in the application)	1

° Special categories of cited documents : [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
04-10-1991	07. 11. 91
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	[illegible]